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Observations on the reproductive period of the freshwater mussel *Potomida littoralis* (Unionidae)

Observaciones sobre el periodo reproductor del mejillón de agua dulce *Potomida littoralis* (Unionidae)

Karl-Otto NAGEL*

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ABSTRACT

Data on the occurrence of gravid *Potomida littoralis* are presented from museum collections, field samplings and published literature. Gravid animals and released glochidia are recorded to occur between March and October. The smallest gravid animals were four or five years old. The length of the brooding period apparently follows a latitudinal cline. *Potomida littoralis* is most likely a tachytictic consecutive brooder with unsynchronised broods.

RESUMEN

Se aportan datos sobre la presencia de ejemplares grávidos de Potomida littorales, a partir de colecciones de museos, muestreos de campo y bibliografía. Los reproductores y la liberación de gloquidios se produce entre marzo y octubre. Los reproductores menores eran animales de cuatro o cinco años de edad. La duración del periodo de cría sigue, en apariencia, una tendencia latitudinal. Potomida littoralis se reproduce en primavera y en verano, con puestas no sincronizadas.

KEY WORDS: Reproduction, Mollusca, *Potomida littoralis*, Unionidae
PALABRAS CLAVE: Reproducción, moluscos, *Potomida littoralis*, Unionidae.

INTRODUCTION

Freshwater mussels (Unionoida) are faced with a serious decline in both their numbers and distribution in many parts of the world (BOGAN, 1993). Reproduction biology and ecology of juvenile mussels are key factors that determine the survival abilities of freshwater mussel populations (BAUER, 2001). Several species occurring in Europe that are more seriously threatened have received considerable attention in the past decades (*Margaritifera margaritifera*: BAUER, 1988; YOUNG, COSGROVE AND HASTIE, 2001; *Unio crassus*: HOCHWALD, 2001; *Pseudunio auricularius* (syn: *Margaritifera auricularia*): ARAUJO AND RAMOS, 2001). The biology and ecology of most other European species remain insufficiently known.

The occurrence of *Potomida littoralis* (Cuvier, 1798) (authorship of this taxon according to FALKNER, BANK AND VON PROSCHWITZ, 2001 and FALKNER, RIPKEN AND FALKNER, 2002) continues to be

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Figure 1. Orientation map for localities from Table I. F: France; E: Spain; P: Portugal; TR: Turkey; SYR: Syria; IL: Israel.
Figura 1. Mapa de las localidades de la Tabla I. F: Francia; E: España; P: Portugal; TR: Turquía; SYR: Siria; IL: Israel.

widespread in Western Europe (France, Spain, Portugal), however for other parts of the species' distribution range (Greece, Turkey, Near and Middle East, Northern Africa) no information is currently available. Data on the general biology of this species date back to the beginning of the last century. HAAS (1917) discovered that the species has 4 marsupial gills (= tetrageny). He examined the anatomy of a single male specimen from France and 11 males and 3 sterile (= non-gravid) females *of Rhombunio rothi* Bourguignat, 1863 from Jaffa/Tel Aviv, Israel (HAAS, 1920). Later, he considered this species to be a synonym of *Potomida littoralis* (HAAS, 1969). It might be noted that the material of *R. rothi* studied by Haas should still be present in the Senckenberg Museum, Frankfurt/M., Germany. Lot n. SNF 000811 contains 25 specimens preserved in alcohol, none of which are gravid. The label reads: "*Rhombunio rothi* (Bgt) - Jaffa – J. Aharoni S. 1913". HAAS (1924) later examined the condition of the marsupial gills of animals collected in Spain. He probably determined the sex of these specimens by histological studies but this is not stated explicitly in his paper. His findings however suggest that *Potomida littoralis* is dioeceous.

This paper is a report on the occurrence of gravid specimens of *P. littoralis* in the river Ognon (eastern France) and on the presence of gravid mussels and glochidia of this species based on published data and museum material. The data is culled from three distinct areas within the species' distribution range.

MATERIAL AND METHODS

Sampling localities and collection dates are given in Table I. All localities are shown on an orientation map (Fig. 1). Data on gravid specimens were obtained from examination of the following collections:

1. Collection Kinzelbach, University of Rostock, Germany (Prof. Dr. R. Kinzelbach). Reference numbers in Table II: VO-78/

Table I. List of localities.
Tabla I. Lista de localidades.

No.	Sampling locality	River or stream	River basin	Departmement, Province, country
France				
1	Cirey-les-Bellevaux , 19 km NNE of Besançon	Ognon	Rhône	Haute-Saône
2	Marnay, ca 20 km WNW of Besançon	Ognon	Rhône	Haute-Saône
3	canal near Montfrin, 20 km WSW of Avignon	Gardon	Rhône	Gard
4	Clerey, 13 km SE of Troyes	Seine	Seine	Aube
5	le Guétin, about 1,5 km upstream of confluence with Loire, 9 km SW of Nevers	Allier	Loire	Nièvre
6	Brehemont, 27 km WNW of Tours	Indre	Loire	Indre-et Loire
7	Candes-St. Martin, 50 km WNW of Tours	Vienne	Loire	Indre-et Loire
8	Chauvigny, 9 km E of Poitiers	Vienne	Loire	Vienne
Spain and Portugal				
9	Lake of Bañolas/Banyoles, ca 16 km NNW of Geron/Girona	endorheic		Girona
10	Acequia (ditch) de San Adrián, ca 44 km ESE of Logroño		Ebro	Navarra
11	Zaragoza	Canal Imperial de Aragón	Ebro	Zaragoza
12	Sástago, app. 56 km SE of Zaragoza	Ebro	Ebro	Zaragoza
13	near Ruidera, ca 26 km SSE of Tomelloso	Lagunas de Ruidera	Guadiana	Albacete and Ciudad Real
14	Goyán/Goián, 12 km upstream of the river mouth at La Guardia/A Guarda (at 3 different but very close sites)	Miño	Miño	Pontevedra
15	near Vila Nova de Cerveira (opposite of Goyán), ca 12 km upstream of the river mouth	Minho	Minho	Viana do Castelo, Portugal
16	Tuy/Tui, 27 km upstream of the river mouth	Miño	Miño	Pontevedra
17	Salvaterra do Miño, 39 km upstream of the river mouth	Miño	Miño	Pontevedra
Turkey, Syria and Israel				
18	near Samanda_i, ca 25 km SW of Hatay (Antakya)	Orontes	Orontes	Hatay, Turkey
19	(1) confluence of the two outlets of the Lake of Homs (Buhairat al-Qattina or B. al-Hims) and (2) backwater	Orontes	Orontes	Syria
20	at bridge of road Homs-Tartus	Orontes	Orontes	Syria
21	stagnant backwater in the al-Ghab ENE of Ain al-Kurum	Orontes	Orontes	Syria
22	canal at crossing of the road Ain al-Kurum to Saqalibiya (Sqelbié)	Orontes	Orontes	Syria
23	Lake Tiberias (Kinneret)		Jordan	Israel

2. Collection of recent freshwater invertebrates at the Museo Nacional de Ciencias Naturales, Madrid, Spain. Reference numbers in Table II: MNCN FW-

3. Senckenberg-Museum, Frankfurt am Main, Germany. Reference numbers in Table II: SNF-

Gills of living or preserved specimens were inspected to see whether they were filled. The developmental status of the content of the gills was not always determined and is not reported here. Live adult mussels were gently opened by hand to approximately 1 cm. The soft parts of most museum specimens were already removed from the shell and separately stored. It is important to remember that in *Potomida littoralis* the inner and outer gills become filled with eggs. In this case they are swollen and non-transparent. Filled marsupia are cream white to light brown in live animals, while unfilled gills are semi-transparent, thin and greyish colour.

Mussels from the river Ognon at Cirey-les-Bellevaux were repeatedly inspected by hand while wading on the riverbanks. The maximum depth sampled was 1.5 m. Sampling dates are given in Table III. The mussels were painted with numbers for individual recognition and measured to the nearest 1/10 mm with a veneer calliper.

RESULTS

Table II reports the findings from collections and field observations. All gravid specimens were encountered from April to October.

The smallest gravid specimen was 39.3 mm in length (June 6, 1990, river Miño at Goyán). The smallest gravid mussel from the Ognon measured 41.0 mm (Table III). In both cases, three, perhaps four, darkly coloured growth lines were evident on the outside of the shell. This means that the animals were in their fourth (or fifth) spring/summer period when they reproduced.

Table III reports the findings of repeated observations of marked individual mussels in one locality in eastern France. Mussel with length 55.3 mm was not found gravid in the year 2000 and became gravid in 2001. Since the animal was among the largest in that population it was most likely also sexually mature in the first year of observation. This individual may have paused from reproduction in 2000 or may have reproduced exceptionally late in that year.

Excluding animals that are less than 39.3 mm, which was the size of the smallest gravid individual, the number of mussels found gravid in the river Ognon was 33%. Similar values were found for the pooled samples from the river Orontes (33%) and the Ebro at Sástago on July 18, 1916 (30%).

DISCUSSION

Little is known about the brood behaviour of unionoidean bivalves. In many cases inferences from circumstantial observations and the lack of distinction between individual and population behaviour have prevented us from detecting the actual patterns until recently (HEARD, 1998). Specific information on the reproductive period of *Potomida littoralis* is scattered. The only published records of gravid animals are from HAAS (1917, 1920) and PRASHAD (1919).

HAAS (1917) conducted regular samplings between July 18 and September 5, 1916 in the river Ebro at Sástago. He found individuals carrying eggs and larvae at the beginning of this period and he recognized freshly metamorphosed mussels on the last date. However, Haas obviously failed to account for the presence of gravid animals in a sample he had taken the year before (September 7-8, 1915; Haas 1916). A small proportion of those mussels, still preserved in the Senckenberg Museum, are gravid (Table II). For the other samples carried out in 1916 (Sástago: August 15, 22, 29, September 5; Gallur, ca 42 km NW of Zaragoza: September 3) Haas did not give the number of *P. littoralis* collected. It remains unclear whether he inspected them for gravidity at all.

PRASHAD (1919) made observations on 3 specimens of *Unio (Rhombunio) semirugatus* collected in October, 1912, in Lake Tiberias (Sea of Galilee). The species name is considered by HAAS (1969) to be a synonym of *Potomida littoralis*. One mussel was gravid in the sample of Prashad. Apparently, it contained glochidia only in the outer gills. In fact, Prashad regarded the outer gills as the only structure having marsupial function (= ectobranchy). Apart from the presence of larvae, Prashad based his identification of marsupial gills on the spacing of the gill septa. HAAS (1924) expressed doubts about the validity of this interpretation since he identified four marsupial gills in *Rhombunio rothi* by histological examinations (HAAS, 1920, 1924). Considering all observations reported in Table II, it might be concluded that the specimen of Prashad was collected at the end of

Table II. Observations on the brooding behaviour of *Potomida littoralis*. The number of gravid mussels when present is given in parenthesis after the sample size. Live glochidia: data from ARAUJO, BRAGADO AND RAMOS (2000). Abbreviations. ?: number not specified.
Tabla II. Observaciones sobre el comportamiento de cría de Potomida littoralis. *El número de ejemplares reproductores se da entre paréntesis tras el tamaño de muestra. Gloquidios vivos: datos tomados de Araujo, Bragado y Ramos (2000). Abreviaturas. ?: número sin específicar.*

Month	Live glochidia	Gravid mussels	Sample size	No. of sampling locality (from Table I)	Date	Reference
January	No[1]					
February	No data	No	2	18	26.2.2001	MNCN FW-681
March	Yes[2]	No	21	11	5.3.1915	Haas, 1916; SMF 003052
April	Yes	No	6	14	16.4.1991	MNCN FW-691
		No	9	1	23.4.2000	this study, see Table III
		Yes	10 (3)	1	29.4.2001	this study, see Table III
May	Yes					
June	Yes	Yes	3 (1)	15	6.6.1990	MNCN FW-640
		Yes	36 (9)	1	10/11.6.2000	this study, see Table III
		Yes	"several" (4)	2	23.6.2000	this study
		Yes	6 (2)	1	23.6.2000	this study, see Table III
		No	20	7	30.6.1991	Nagel and Badino, 2001
		No	18	6	30.6.1991	Nagel and Badino, 2001
July	Yes	No	33	4	1.7.1991	Nagel and Badino, 2001
		Yes	1 (1)	10	11.7.1996	MNCN FW-1157
		No	7	14	11.7.1990	MNCN FW-645
		Yes	"several hundred" (?) sub-sample: 47 (14)	12, at 0-0.5m depth	18.7.1916	Haas, 1917, 1920; SMF 003054; this study
		No	5	5	21.7.1985	Nesemann and Nagel, 1989
		No	3	6	23.7.1985	Nesemann and Nagel, 1989
		No	4	7	24.7.1985	Nesemann and Nagel, 1989
		No	2	8	26.7.1985	Nesemann and Nagel, 1989
August	Yes	Yes	"many" (2) at 0-0.5 m , "some" (all ?) at 5-6 m depth	12	2.8.1916	Haas, 1917; Haas, 1920
		No	6	21	3.8.1978	VO-78/12
		Yes	more than 1 (1)	22	3.8.1978	VO-78/12a
		Yes	8 (4)	23	5.8.1978	VO-78/20
		Yes	17 (6)	24 and 25	8/10.8.1978	VO-78/28; VO-78/29
		No	8	18	4.8.1988	MNCN FW-1295
		Yes	? at 0 -0.5 m, ? (all ?) at 6 m depth	12	8.8.1916	Haas, 1917
		Yes	9 (2)	20	23.8.1978	VO-78/49
		No	2	14	28.8.1990	MNCN FW-652
September	Yes	Yes	sub-sample: 35 (5)	12	7/8.9.1915	SMF 323165; this study; (Haas, 1916)
		No	42	12	8.9.1915	Haas, 1916; SMF 003053
		No	14	13	8.9.2000	MNCN FW-1500
		No	1	19	11.9.2002	MNCN FW-1543
		No	10	9	15.9.1914	SMF 003049
		No	5	14	18.9.1990	MNCN FW-654
October	Yes[3]	Yes	3 (1)	23	October 1912	Prashad, 1919
		No	17	3	5.10.1989	Nagel and Badino, 2001
		No	19	17	7.10.1988	Nagel and Badino, 2001
		No	1	16	24.10.1989	MNCN FW-498
November	No data	No	2	14	6.11.1990	MNCN FW-664
		No	1	18	6.11.1990	MNCN FW-665
December	No					

[1] four dates in 1998; [2] from 6.3.1997; [3] until 9.10.1997

Table III. Observations of the reproductive status of Potomida littoralis in the river Ognon near Cirey-les-Bellevaux, France. From a total of 37 individually marked animals only those are listed that could be inspected at least two times. The remainding ones were not gravid on April 23, 2000 (1 specimen) or on June 10 - 11, 2000 (12 specimens). Length of specimens as measured on day of first record. No intensive sampling on June 23, 2000.

Tabla III. Observaciones del estatus reproductor de Potomida littoralis *en el río Ognon cerca de Cirey-les-Bellevaux, Francia. De un total de 37 especímenes marcados sólo se incluyen aquellos que se observaron al menos dos veces. Los restantes no estaban en estado reproductor el 23 de abril de 2000 (1 ejemplar) o el 10-11 de junio de 2000 (12 ejemplares). Se da la longitud tomada el día de la primera observación. No se realizó un muestreo intenso el 23 de junio de 2000.*

Length (mm)	Gravid 2000 April 23	Gravid 2000 June 10/11	Gravid 2000 June 23	Gravid 2001 April 29	Length (mm)	Gravid 2000 April 23	Gravid 2000 June 10/11	Gravid 2000 June 23	Gravid 2001 April 29
27.8		-	-		47.0		-	+	-
30.2		-	-		47.7		+		
39.0		-		-	48.1		-		-
39.4		-		-	48.2		+		
40.0	-	-			49.3		+	+	
41.0		-		+	50.2	-	-		
41.8		+			51.5	-	+		-
44.6		+		+	51.7	-	+		
45.6	-	-			54.7		-	-	-
46.6		-		-	55.3	-	-	-	+
46.7		+			58.7	-	+		
46.8	-	-			65.9		-	†	

Explanation of symbols: + gravid, - not gravid, † dead, no entry: not found.

the gravidity period and had already expelled the content of the inner gills thus giving the appearance of an ectobranchous state.

Based on the observations of HAAS (1920, 1924), NAGEL (1988, p. 3, fig. 1) hypothesized that *Potomida littoralis* is a summer breeder, i.e. tachytictic like the European species of the genus *Unio*. The observations from the river Ognon (Table III) fit well into this pattern. Recently, ARAUJO, BRAGADO AND RAMOS (2000) reported on live glochidia (valves with soft body) in drift net samples from early March to beginning of October, 1997, in the Canal Imperial de Aragón that takes water from the Ebro (sampling localities near the villages of Grisén and Alagón, between Gallur and Zaragoza, see Table II). These data confirm the tachitixis but also give evidence of a very long reproductive period.

No gravid mussels were found in France in the 64 specimens sampled in July and October. Several explanations are possible for this, for example, latitude. It may be safe to assume that the reproductive season for *P. littoralis* is abbreviated at higher latitudes where periods with water temperatures favourable for the growth of food particles (bacteria, algae) are short. There is evidence that at least some unionoid species allocate only surplus energy to reproduction (HOCHWALD, 2001 and references therein). Alternative explanations are population specific and would point to a high parasite load and natural or anthropogenic habitat disturbances. All these factors can induce individuals or entire populations to suppress reproduction. This latter phenomenon was previously described for some species (*Margaritifera margaritifera*: BAUER, 1987; ROSS, 1992; *Unio crassus*: HOCHWALD,

2001; *Hyridella depressa*: WALKER, BYRNE, HICKEY AND ROPER, 2001).

The apparently very long reproductive period in the southern locations makes consecutive breeding (more than one brood in the reproductive period) of the species quite probable, but presently there is no direct evidence for this. This form of reproductive behaviour, however, seems to be widespread among unioniod mussels (HEARD, 1998).

A mean of 30% gravid mussels was found in the samples from the Ognon, one sample from the Ebro and the pooled Orontes samples (Table II). Assuming a sex ratio of 1:1 would mean that one-third of the females did not actively participate in reproduction at the times of sampling. This pattern indicates unsynchronised breeding within a population.

From the above it is concluded that *Potomida littoralis* is a tachytictic brooder with probably consecutive unsynchronised broods. The reproductive period can span from March to October. More data are needed to determine if the length of the reproductive period follows a latitudinal cline.

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La malacofauna de la Sierra de Alcaraz (Albacete, España)

The molluscan fauna of the Alcaraz mountains (Albacete, Spain)

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RESUMEN

Se realiza el primer estudio de la malacofauna continental de la sierra de Alcaraz (Albacete, España). Los datos previos son escasos, dispersos y de muy diversos autores. Se han recogido muestras en 29 localidades del área de estudio y se han hallado un total de 50 especies de gasterópodos, de las cuales 47 son terrestres y 3 dulceacuícolas. Se citan por primera vez 22 especies en la provincia de Albacete y 10 en la Comunidad de Castilla-La Mancha.

ABSTRACT

The first complete study of non-marine molluscs of the Alcaraz mountains (Albacete, Spain) has been carried out. Previous data are scarce, disperse and from many diverse authors. Samples have been collected in 29 localities of the study area and a total of 50 species of gastropods have been found, 47 corresponding to land species and 3 to freshwater species. Twenty-two species are recorded for the first time for the province of Albacete and 10 for the "Comunidad de Castilla-La Mancha".

PALABRAS CLAVE: moluscos, terrestres, dulceacuícolas, Alcaraz, Albacete, España.
KEY WORDS: molluscs, land, freshwater, Alcaraz, Albacete, Spain.

INTRODUCCIÓN

La sierra de Alcaraz, con una extensión de 156.000 hectáreas, se encuentra en el SW de la provincia de Albacete y enlaza con las sierras andaluzas de Cazorla y Segura. Junto a la cabecera del río Mundo forma parte del "Parque Natural de la Sierra de Alcaraz y Alto del Segura" de la Comunidad Autónoma de Castilla-La Mancha. Hasta ahora se han realizado algunos inventarios de fauna (ANDÚJAR TOMÁS Y GÓMEZ DE GUEVARA, 1985; VIDAL-ABARCA, SUÁREZ, MILLÁN, GÓMEZ, ORTEGA, VELASCO Y RAMÍREZ-DÍAZ, 1991) y de flora (HERRANZ Y GÓMEZ CAMPO, 1986; GUERRERA MONTES, ROS ESPÍN, HERAS IBÁÑEZ, GARCÍA ZAMORA Y JIMÉNEZ MARTÍNEZ, 1989) de esta sierra, pero se poseen escasos datos sobre su malacofauna.

Esta falta de información se extiende a la mayor parte de la provincia de Albacete, en la cual predominan las citas aisladas de diversos autores, destacando

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Figura 1. A. Situación geográfica del área de estudio (gris: provincia de Albacete; negro: sierra de Alcaraz). B. Localización de las localidades de muestreo en la sierra de Alcaraz (Albacete).
Figure 1. A. Geographical situation of the study area (grey: Albacete province; black: Alcaraz mountains). B. Location of the sampled localities in the Alcaraz mountains (Albacete).

entre ellas la descripción de una nueva especie, *Helix semipicta*, por HIDALGO (1870), cuya localidad tipo es Alcaraz.

En los últimos 30 años han aumentado las referencias sobre la malacofauna de la provincia de Albacete. GASULL (1975) cita varias especies, y RAMOS Y APARICIO (1985) estudian los gasterópodos terrestres y dulceacuícolas de las Lagunas de Ruidera, encontrando un total de 19 especies. ROBLES, BORREDÀ Y COLLADO (1991) estudian los moluscos continentales de la región de Almansa, y citan 43 especies. BORREDÀ Y COLLADO (1991) y BORREDÀ, COLLADO, BLASCO Y ESPÍN (1991) estudian los pulmonados desnudos de la provincia, citando un total de 11 especies. Los moluscos dulceacuícolas también han sido objeto de estudio, sobre todo en el río Júcar: JIMÉNEZ Y MARTÍNEZ-LÓPEZ (1988) determinan 9 especies, MARTÍNEZ-LÓPEZ, PUJANTE Y TAPIA (1993) siete, y RUEDA, HERNÁNDEZ Y TAPIA (2001) nueve especies. Además, VIDAL-ABARCA *ET AL.* (1991) realizan un estudio limnológico de la cuenca del río Mundo, donde citan 11 especies.

En su Tesis Doctoral, PUENTE (1994) realiza una revisión de los Helicoidea de la Península Ibérica y cita algunas especies procedentes del área de estudio. BORREDÀ (1996) caracteriza las babosas del este de la Península Ibérica y cita varias especies en la provincia de Albacete, algunas de ellas en el área de muestreo del presente trabajo. Por último, GÓMEZ Y DANTART (1996) dan a conocer nuevas localidades de especies del Orden Orthurethra recolectadas en Castilla-La Mancha y, algunas de ellas, en la provincia de Albacete.

En este trabajo se da a conocer el primer inventario detallado de las especies de moluscos terrestres y dulceacuícolas de la sierra de Alcaraz, lo que permite ampliar el conocimiento de este filo en la provincia de Albacete y en la Comunidad Autónoma de Castilla-La Mancha.

Tabla I. Listado de las localidades de muestreo en la sierra de Alcaraz (Albacete, España). Abreviaturas: N, número de la localidad de muestreo.
Table I. List of the sampled localities in Alcaraz mountains (Albacete, Spain). Abbreviations: N, number of the sampling locality.

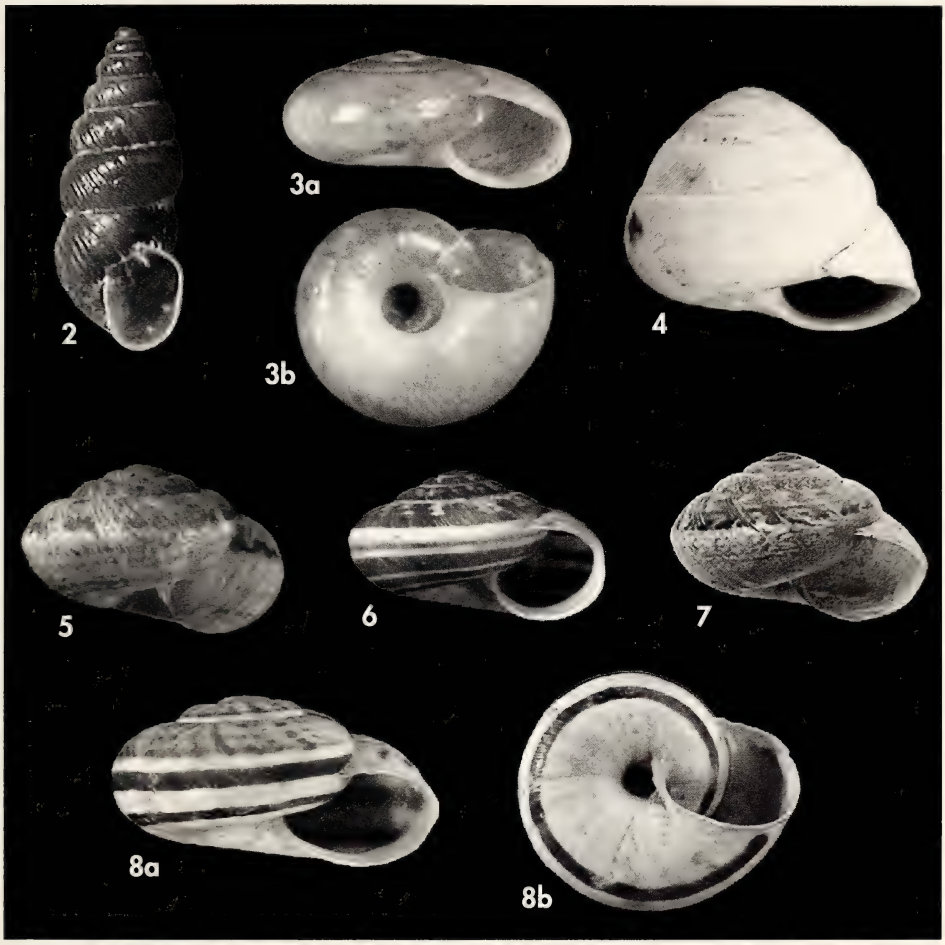
N	Localidad	Fecha	U.T.M.	Altitud	Hábitat
1	Ermita Cortés. Depósito de agua	10.10.96	30SWH4683	1100	Carrascal.
2	La Hoz. Río Cortes	10.10.96	30SWH4882	1040	Nogales/Ribera
3	Peñascosas. Camping río del Arquillo	10.10.96	30SWH5180	1140	Chopera/ Carrascal
4	Las Baguadas	11.10.96	30SWH5379	1180	Pinada/Quejigal/Juncal
5	Casas de Carboneras	11.10.96	30SWH5679	1300	Pinada
6	Nacimiento del río del Vidrio	11.10.96	30SWH6077	1240	Ribera
7	Rambla de la Dehesa del Val	11.10.96	30SWH6474	900	Retamar/Ruderal
8	Río de Alcaraz. Molino de Potrera	12.10.96	30SWH4379	980	Ribera/ Chopera
9	Vianos. Cabecera del Bco. de los Quiñones	12.10.96	30SWH4376	1100	Carrascal
10	Carretera de Vianos, km 158,5	12.10.96	30SWH4573	1220	Arroyo
11	Reolid. Camino al Balneario. Río Salobre	15.03.97	30SWH3774	840	Ribera/Sauceda
12	Cerro de los Pizorrosos del Aljibe	15.03.97	30SWH3672	1000	Matorral/ Carrascal
13	Salobre. Río Salobre	15.03.97	30SWH3971	940	Ribera
14	La Herrería	15.03.97	30SWH4170	980	Pinada
15	Zapateros, a 500 m	16.03.97	30SWH4367	1100	Ribera/Chopera
16	Subida al Pico Almenaras	16.03.97	30SWH4665	1380	Carrascal/Pinada
17	Pico Almenaras	16.03.97	30SWH4866	1798	Pinada/Veget. pratense
18	Puerto del Mosquito	16.03.97	30SWH4968	1600	Pinada/Carrascal
19	Cruce de carreteras	17.03.97	30SWH5070	1440	Pinada/Carrascal
20	Puerto de Crucetas	17.03.97	30SWH4363	1300	Pinada
21	Arroyo de las Fábricas	17.03.97	30SWH4362	1200	Pinada/Ribera
22	Cortijo de Vilutia	17.03.97	30SWH4764	1180	Pinada/Carrascal / Matorral
23	Las Espineras	17.03.97	30SWH5165	1380	Roquedo/Arroyo
24	Entre Batán del Puerto y Río Madera	18.03.97	30SWH5970	1250	Pinada/Roquedo
25	Río Motilla	18.03.97	30SWH5873	1040	Ribera
26	La Casa Nueva	18.03.97	30SWH5974	1080	Pinada/Matorral
27	Nacimiento Río Mundo	18.03.97	30SWH4956	1040	Ribera/Roquedo
		21.04.97			
28	Río Arquillo (col. R. Carr)	02.06.02	30SWH5589	1000	Ribera
29	Riópar (col. R. Carr)	01.06.02	30SWH5061	980	Ribera

MATERIAL Y MÉTODOS

El área de estudio engloba la sierra de Alcaraz (Fig. 1), donde se han seleccionado 27 localidades extendidas por la mayor parte de su área geográfica (Fig. 1b; Tabla I). La duración mínima de cada muestreo ha sido de una hora y se ha muestreado en los diferentes ambientes presentes en cada localidad. Para la localización de las estaciones se han utilizado los mapas topográficos a escala 1:50.000. Cada estación se indica por sus coordenadas UTM con precisión de 1x1 km, así como por su altitud y su hábitat (Tabla I). De la colección Ron Carr se han estudiado muestras de 3 localidades; dos de ellas, 28 y 29, no figuraban entre nuestras localidades de muestreo, por lo que se han incorporado al listado, mientras que la tercera corresponde a nuestra localidad 27.

Tabla II. Listado de especies de moluscos continentales de la Sierra de Alcaraz (Albacete, España) (primer número: ejemplares recolectados vivos/segundo número: conchas).
Table II. List of the non-marine molluscs of the Alcaraz mountains (Albacete, Spain) (first number: alive collected specimens/ second number: shells).

ESPECIES	LOCALIDADES																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Potamopyrgus antipodarum (J. E. Gray, 1843)		2/5		6/0		17/0		50/0			5/0										1/0				6/0				
Stagnicola sp.						0/1																							
Ancylus fluviatilis O. F. Müller, 1774						2/0					3/0														3/0		4/0		
Carychium (Saraphia) tridentatum (Risso, 1826)						2/0							0/1		7/0										2/0				
Oxyloma (Oxyloma) elegans elegans (Risso, 1826)						2/3																						1/0	1/0
Cochlicopa lubrica (O. F. Müller, 1774)													0/2		0/4														
Lauria (Lauria) cylindracea (Da Costa, 1778)			2/0	1/0						2/6	0/2		0/3																
Vallonia costata (O. F. Müller, 1774)													0/1		3/1		0/2	0/2		0/12	0/6			0/1					
Vallonia enniensis (Gredler, 1856)															7/0														
Vallonia pulchella (O. F. Müller, 1774)															4/1										2/0				
Acanthinula aculeata (O. F. Müller, 1774)													0/6																
Pyramidula pusilla (Vallot, 1801)																					12/1		7/0	0/2			5/0		
Granopupa granum (Draparnaud, 1801)									1/0													0/15							
Chondrina granatensis Alonso, 1974																							14/0	10/3			2/6		
Truncatellina callicratis (Scacchi, 1833)													0/2											0/2					
Truncatellina claustralis (Gredler, 1856)																						0/2							
Jaminia quadridens (O. F. Müller, 1774)			0/3		0/3				2/4					0/2								0/3				0/1			
Merdigera obscura (O. F. Müller, 1774)			0/4		0/4						1/4		0/1																
Cecilioides (Cecilioides) acicula (O. F. Müller, 1774)	2/0												0/1																
Ferussacia (Ferussacia) folliculus (Gmelin, 1791)	1/2											0/5																	
Rumina decollata (Linnaeus, 1758)	0/1							0/1	0/3			0/1	0/1	0/2															
Paralaoma servilis (Shuttleworth, 1852)				0/1						0/5			0/2						0/2	0/1	0/3	0/2		0/2					
Discus rotundatus (O. F. Müller, 1774)								8/0																					
Vitrea (Crystallus) contracta (Westerlund, 1871)	1/0		1/0	2/1	0/1						1/0		0/1		0/6			0/1	0/1	1/3	3/8	1/1	0/1	0/8					
Euconulus (Euconulus) fulvus (O. F. Müller, 1774)																					0/1								
Oxychilus (Oxychilus) draparnaudi draparnaudi (H. Beck, 1837)		3/1	1/0					1/0			0/2		3/2		1/0												2/0		
Aegopinella nitidula (Draparnaud, 1805)										4/11																			
Milax (Milax) gagates (Draparnaud, 1801)			3					2					3																
Vitrina pellucida pellucida (O. F. Müller, 1774)																				1/0	0/2								
Oligolimax annularis (Studer, 1820)					1/1																			0/1					
Lehmannia valentiana (A. Férussac, 1823)		1				2					1		2																
Deroceras (Deroceras) laeve (O. F. Müller, 1774)		3													2								2						
Deroceras nitidum (Morelet, 1845)					2																						2		
Deroceras (Deroceras) reticulatum (O. F. Müller, 1774)	2	1	3			2		1		2	2									2		1	1		2		2		
Sphincterochila (Cariosula) baetica (Rossmässler, 1854)									0/2																				
Arion (Arion) lusitanicus J. Mabille, 1868		1		2																			1				3		
Cochlicella (Prietocella) barbara (Linnaeus, 1758)						0/1		2/0			0/5																		
Monacha (Monacha) cartusiana (O. F. Müller, 1774)		1/1																											
Xerocrassa subrogata (Rossmässler in Pfeiffer, 1853)	1/0		1/5		7/8									4/7		0/2						0/15				0/5			
Xerocrassa penchinati (Bourguignat, 1868)																				0/7									
Xerotricha conspurcata (Draparnaud, 1801)	2/4																												
Helicella cistorum (Morelet, 1845)												1/14																	
Candidula gigaxii (Pfeiffer, 1850)				0/21			1/0						1/0		1/0							0/1							
Cernuella (Cernuella) virgata (Da Costa, 1778)			3/4				5/5	2/0			0/4		0/1										0/3				1/0		
Xerosecta (Xerosecta) cespitum (Draparnaud, 1801)	5/0	3/1	0/4				2/4																1/2				4/0		
Microxeromagna lowei (Potiez y Michaud, 1838)		2/1							0/3		9/6																		
Cepaea (Cepaea) nemoralis nemoralis (L., 1758)				0/9						0/2	0/1				0/1						0/4		0/1				0/1		
Cornu aspersum (O. F. Müller, 1774)		1/1	2/0			0/2	0/1					0/2	1/1	0/1	1/0						0/2		1/0						
Iberus gualtierianus (L., 1758) m. guiraoanus (Rossmässler, 1854)														0/3			1/52	0/2			1/12		0/1	0/2			0/20		
Theba pisana (O. F. Müller, 1774)									0/3																				



Figuras 2-8. Algunas especies interesantes de moluscos recolectadas en la sierra de Alcaraz (Albacete). 2. *Chondrina granatensis* (localidad 24) (diámetro, 2,7 mm). 3. *Aegopinella nitidula* (loc. 10) (diámetro, 7,1 mm). 4. *Sphincterochila baetica* (loc. 9) (diámetro, 20,05 mm). 5. *Xerocrassa penchinati* (loc. 20) (diámetro, 4,2 mm). 6. *Candidula gigaxii* (loc. 13) (diámetro, 7,8 mm). 7. *Helicella cistorum* (loc. 12) (diámetro, 11,1 mm). 8. *Iberus gualtierianus guiraoanus* (loc. 21) (diámetro, 24,85 mm). *Figures 2-8. Some interesting species of molluscs collected in the Alcaraz mountains (Albacete). 2. Chondrina granatensis (loc. 24) (diameter 2.7 mm). 3. Aegopinella nitidula (loc. 10) (diameter 7.1 mm). 4. Sphincterochila baetica (loc. 9) (diameter 20.05 mm). 5. Xerocrassa penchinati (loc. 20) (diameter 4.2 mm). 6. Candidula gigaxii (loc. 13) (diameter 7.8 mm). 7. Helicella cistorum (loc. 12) (diameter 11.1 mm). 8. Iberus gualtierianus guiraoanus (loc. 21) (diameter 24.85 mm).*

El listado de las especies determinadas se muestra en la Tabla II. Se han realizado estudios anatómicos del aparato reproductor de aquellas especies cuyos caracteres conquiliológicos son insuficientes para identificarlas correctamente. Para la determinación taxonómica y posición sistemática se han tenido en cuenta los trabajos de ALTONAGA (1988), GÓMEZ (1988), FECHTER Y FALKNER (1993), PUENTE (1994), BORREDÀ (1996), GLÖER Y MEIER-BROOK (1998), MARTÍNEZ-ORTÍ (1999), BANK, FALKNER, NORDSIECK Y RIPKEN (2001) y FALKNER, RIPKEN Y FALKNER (2002), entre otros. Todo el material recolectado se

encuentra depositado en el Museu Valencià d'Història Natural.

RESULTADOS Y DISCUSIÓN

Se han hallado un total de 50 especies de moluscos, pertenecientes a 14 superfamilias y 27 familias, de las cuales 47 corresponden a gasterópodos terrestres y tres a dulceacuícolas. Las especies de moluscos encontradas en el área de muestreo aparecen relacionadas en la Tabla II.

Comentarios taxonómicos: La taxonomía del género *Stagnicola* Jeffreys, 1830 ha sido revisada recientemente por diversos autores (BARGUES, VIGO, HORAK, DVORAK, PATZNER, POINTIER, JACKIEWICZ, MEIER-BROOK Y MAS-COMA, 2001, con referencias; FALKNER *ET AL.*, 2002, con referencias). En la actualidad, se acepta que este género está representado en Europa por cinco especies, de las que sólo una, *S. fuscus* (Pfeiffer, 1821), ha sido caracterizada en España, mediante análisis molecular y estudios anatómicos. La ausencia de partes blandas en el único ejemplar recogido (una concha juvenil), impide su asignación a una especie concreta del género *Stagnicola*.

ROBLES, BORREDÀ Y COLLADO (1991) citan *Carychium* sp. en la región de Almansa. La revisión de este material permite asignarlo a *C. tridentatum* (Risso, 1826).

Helix semipicta Hidalgo, 1870, cuya localidad tipo es la sierra de Alcaraz, es un sinónimo posterior de *Helicella cistorum* (Morelet, 1845) (MARTÍNEZ-ORTÍ Y APARICIO, 2003). Esta última especie es un endemismo ibérico (PUENTE, 1994) que se extiende por el Alemtejo (localidad tipo) y el este del Algarve (Portugal) y por las provincias españolas de Badajoz y Cáceres, suroeste de Madrid y de Toledo, Ciudad Real, Córdoba, Jaén, Huelva y Sevilla. Por lo tanto, su hallazgo constituye la primera cita en la provincia de Albacete, siendo además, la más oriental para esta especie (MARTÍNEZ-ORTÍ Y APARICIO, 2003).

La composición específica del género *Iberus* Montfort, 1810 es controvertida. Los autores más recientes (PUENTE, 1994; ARRÉBOLA, 1995) consideran que existe una sola especie muy polimórfica, *I. gualtierianus* (Linnaeus, 1758), criterio que seguimos aquí. No obstante, hay que tener en cuenta que otros autores (APARICIO, 1983; APARICIO Y RAMOS, 1988) reconocen la existencia de varias especies bien definidas dentro del género, una de las cuales sería *I. guiraoanus*, si bien APARICIO Y RAMOS (1988) plantean la necesidad de nuevos estudios para resolver los problemas taxonómicos planteados. Actualmente se están realizando análisis moleculares (B. GÓMEZ, com. pers.), que esperamos contribuyan a solventar el problema. *I. guiraoanus* fue descrito originalmente de Castellón por ROSSMÄSSLER (1854), donde no ha vuelto a ser encontrado, y es conocido de las provincias de Granada y Jaén (var. *angustata* Rossmässler, 1854) (GARCÍA SAN NICOLÁS, 1957; ALONSO, 1975; APARICIO, 1983).

ORTIZ DE ZÁRATE (1991) cita *Iberus alcarazanus* (Guirao en Rossmässler, 1854) en la sierra de Alcaraz, pensamos que erróneamente, ya que el morfotipo *alcarazanus* es más globoso y no presenta ombligo. La posesión de un ombligo bien conformado es la principal característica del morfotipo *guiraoanus*.

Comentarios biogeográficos: Desde el punto de vista biogeográfico podemos agrupar las especies halladas en los siguientes conjuntos, teniendo en cuenta sus áreas de distribución: una especie introducida, de origen indo-australiano: *Potamopyrgus antipodarum*; siete especies holárticas: *Oxyloma elegans elegans, Cochlicopa lubrica, Vallonia costata, V. pulchella, Euconulus fulvus, Vitrina pellucida pellucida* y *Deroceras laeve*; dos paleárticas: *Acanthinula aculeata* y *Ancylus fluviatilis*; cuatro especies de amplia distribución europea: *Carychium tridentatum, Merdigera obscura, Deroceras reticulatum y Aegopinella nitidula*; otras cuatro especies de amplia distribución europea, aunque más restringida: *Vallonia enniensis, Discus rotundatus, Oligolimax annularis y Cepaea nemoralis nemoralis*; doce especies de distribución europea occidental y mediterránea: *Lauria cylindracea, Pyramidula pusilla,*

Truncatellina callicratis, T. claustralis, Jaminia quadridens, Cecilioides acicula, Oxychilus draparnaudi draparnaudi, Monacha cartusiana, Cernuella virgata, Cornu aspersum, Milax gagates y *Candidula gigaxii*; cuatro especies preferentemente mediterráneas: *Ferussacia folliculus, Rumina decollata, Paralaoma servilis* y *Microxeromagna lowei*; otras cuatro especies preferentemente mediterráneas que alcanzan la costa atlántica occidental: *Granopupa granum, Cochlicella barbara, Xerotricha conspurcata* y *Theba pisana pisana*; una especie ibérica cuya área de distribución se prolonga por el SE de Francia: *Xerosecta cespitum*; una especie iberomagrebí: *Sphincterochila baetica*; ocho endemismos ibéricos: *Chondrina granatensis, Xerocrassa subrogata, X. penchinati, Helicella cistorum, Iberus gualtierianus* morfotipo *guiraoanus, Lehmannia valentiana, Deroceras nitidum* y *Arion lusitanicus* (FECHTER Y FALKNER, 1993; ALTONAGA, GÓMEZ, MARTÍN, PRIETO, PUENTE y RALLO, 1994; PUENTE, 1994; BORREDÀ, 1996). Alguna de ellas, como *Lehmannia valentiana* y *Arion lusitanicus* o *Potamopyrgus antipodarum*, que originariamente presentaban una distribución natural ibérica (las dos primeras) o de Nueva Zelanda (la última), se han extendido ampliamente por varios continentes (FECHTER Y FALKNER, 1993; BORREDÀ, 1996).

Esta fauna malacológica presenta una geonemia predominantemente mediterránea con clara influencia continental, como corresponde al área geográfica estudiada. Cuarenta y seis de las 50 especies determinadas son comunes a la Comunidad Valenciana. De las cuatro restantes, *Chondrina granatensis* e *Iberus gualtierianus* morfotipo *guiraoanus* son especies de distribución meridional, que alcanzan en la sierra de Alcaraz el límite norte de su área de distribución, donde se han encontrado por primera vez. *Helicella cistorum* es un endemismo ibérico occidental, correspondiendo la nueva localidad, cerro de Los Pizarrosos, al punto más oriental conocido. Por último, *Candidula gigaxii* presenta distribución discontinua en la Península Ibérica y es conocida en algunos puntos de la provincia de Jaén cercanos a la sierra de Alcaraz.

Conservación: La mayoría de las especies halladas en la sierra de Alcaraz carecen actualmente de medidas de protección a nivel autonómico, estatal o europeo. En general, se trata de especies triviales que presentan una amplia distribución peninsular. Sin embargo, *Iberus guiraoanus* ha sido incluido en el Catálogo Regional de Especies Amenazadas de Castilla-La Mancha (Decreto 33/1998 de 5 de Mayo), con la categoría de "interés especial". El grupo de expertos de la Sociedad Española de Malacología no propone ningún taxón de los hallados en esta región para su inclusión en el Catálogo Nacional de Especies Amenazadas (ALONSO, ALTONAGA, ÁLVAREZ, ARAUJO, ARCONADA, ARRÉBOLA, BECH, BROS, CASTILLEJO, GÓMEZ, IBÁÑEZ, LUQUE, MARTÍNEZ-ORTÍ, MORENO, PRIETO, PUENTE, PUJANTE, ROBLES, ROLÁN Y TEMPLADO, 2001).

CONCLUSIONES

Se han hallado por primera vez veintidós especies en la provincia de Albacete, diez de las cuales (señaladas con un asterisco) se citan por primera vez en la Comunidad de Castilla-La Mancha: *Carychium tridentatum, Cochlicopa lubrica, Lauria cylindracea, Vallonia enniensis, V. pulchella, Acanthinula aculeata* (*), *Pyramidula pusilla, Truncatellina claustralis* (*), *Chondrina granatensis* (*), *Merdigera obscura, Paralaoma servilis* (*), *Discus rotundatus* (*), *Aegopinella nitidula* (*), *Euconulus fulvus* (*), *Oxychilus draparnaudi draparnaudi, Vitrina pellucida pellucida, Oligolimax annularis, Sphincterochila baetica* (*), *Candidula gigaxii, Helicella cistorum, Xerocrassa penchinati* (*) e *Iberus gualtierianus* morfotipo *guiraoanus* (*). Estas nuevas citas amplían el conocimiento sobre la distribución de estas especies en la Península Ibérica.

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Los moluscos de las aguas continentales de la provincia de Huelva (SO España)

Freshwater Molluscs of Huelva Province (SW Spain)

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RESUMEN

En este trabajo se estudia la faunística y distribución de los moluscos de las aguas continentales de la provincia de Huelva (SO de España). Se han encontrado 29 especies de gasterópodos y bivalvos repartidos en cinco cuencas hidrográficas, siendo las especies de mayor abundancia *Physella acuta*, *Ancylus fluviatilis*, *Planorbarius metidjensis*, *Radix balthica* (= *R. peregra*) (Gastropoda) y *Pisidium casertanum* (Bivalvia); todas las especies se referencian en el entramado provincial de cuadrículas UTM 10 x 10 km.

ABSTRACT

In this work we study the faunistic and distribution of freshwater molluscs of Huelva (SW Spain). We have found 29 species of gastropods and bivalves in five hydrographic basins, the most abundant species were *Physella acuta*, *Ancylus fluviatilis*, *Planorbarius metidjensis*, *Radix balthica* (= *R. peregra*) (Gastropoda) and *Pisidium casertanum* (Bivalvia); all the species are refered to the 10 x 10 km UTM grid.

PALABRAS CLAVE: Moluscos, Gasterópodos, Bivalvos, Huelva, Andalucía, España, Distribución, Ríos.
KEY WORDS: Molluscs, Gastropoda, Bivalvia, Huelva, Andalucia, Spain, Distribution, Rivers

INTRODUCCIÓN

La malacofauna continental de la provincia de Huelva ha sido, en general, poco estudiada; mientras que existe un catálogo bastante preciso de las especies terrestres (Muñoz, 1992), de la faunística dulceacuícola sólo se conocen aproximaciones muy generales o localizadas (Ortiz de Zárate y Ortiz de Zárate, 1961; Bigot y Marazanof, 1965; Marazanof, 1966; Gasull, 1985; Pérez-Quintero, 1988), habiéndose citado un máximo de 14 especies en el entorno provincial de las que la mayoría han sido descritas en el entorno de las marismas del Guadalquivir y Coto de Doñana. Recientemente, Montes del Olmo (1993), estudiando el efecto de la introducción de *Procambarus clarkii* (Girard, 1852) en Doñana, encuentra sólo cinco especies en el entorno del Parque Nacional, no existiendo por tanto confirmación de la presencia actual de algunas de las descritas previamente en la zona. Tras un detallado estudio de los cursos

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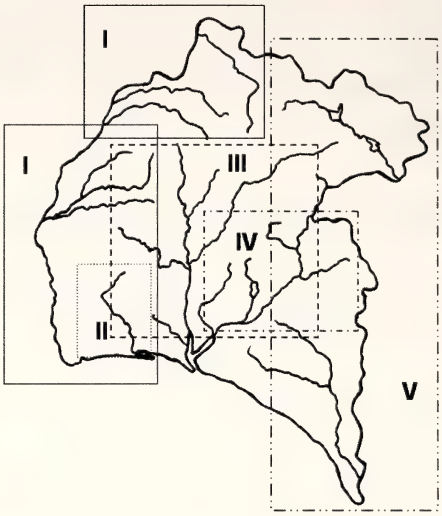

Figura 1. Distribución de las cinco cuencas hidrográficas de la provincia de Huelva: I Guadiana, II Piedras, III Odiel, IV Tinto, V Guadalquivir.
Figure 1. Distribution of the five hydrographic basins of the province of Huelva: I Guadiana, II Piedras, III Odiel, IV Tinto, V Guadalquivir.

de agua de la provincia, en este trabajo se amplía el listado de especies a 29 (21 Gasterópodos y 8 Bivalvos), aportando datos sobre la distribución actual de las mismas en las distintas cuencas hidrográficas de la red fluvial provincial.

MATERIAL Y MÉTODOS

Se han prospectado 251 localidades, habiéndose encontrado moluscos en 228 de ellas (91,0 % de frecuencia de captura): 64 en la cuenca del río Guadalquivir, 40 en la del río Tinto, 48 en la del río Odiel y 76 en la de los ríos Guadiana y Piedras (Fig. 1), que han sido muestreadas entre los años 1999 y 2002, todas ellas en, al menos, dos ocasiones.

En cada estación se registraba la coordenada UTM y la altitud, ambas mediante GPS (ver Anexo); se anotaba, igualmente, la profundidad, densidad y situación espacial de los individuos, así como una estima de la velocidad de la corriente y la granulometría del sustrato. Para cada especie se ha calculado su cobertura como porcentaje de presencia referido a las 127 cuadrículas UTM 10x10 km de la provincia, y su abundancia como porcentaje de presencia referido a las 228 estaciones en que se han encontrado moluscos; en ambos casos siempre se computan los individuos vivos, no sus restos. En cada cuenca se llevaron a cabo dos aproximaciones a su diversidad: consignando el número de especies, géneros y familias (riqueza taxonómica), y calculando su diversidad específica según la expresión de SHANNON-WEAVER: $H= -\Sigma p_i \ln p_i$ (MAGURRAN, 1988); a efectos de cálculo, y considerando la escasa superficie de la cuenca del río Piedras y su proximidad geográfica con la del río Guadiana, se creyó conveniente asociar la primera con la segunda.

Los moluscos se colectaron barriendo el fondo de los cauces con redes de 0,3 mm de luz, y capturándolos manualmente en rocas, plantas y otros sustratos sumergidos. Tras la captura y lavado de muestras los ejemplares fueron conservados en alcohol al 70% y posteriormente determinados a nivel de especie siguiendo las indicaciones de manuales clásicos (ADAM, 1960; ELLIS, 1978) y actuales (GLÖER y MEIER-BROOK, 2003). En algunas especies de difícil determinación a partir de la mor-

fología de su concha, se recurrió al análisis de su aparato reproductor.

RESULTADOS

En el análisis de las cuencas y subcuencas estudiadas se han encontrado 25 géneros y 29 especies, de las que 27 son autóctonas (93,1 %) y 2 introducidas (*Potamopyrgus antipodarum* (J.E. Gray 1843) y *Corbicula fluminea* (O.F. Müller 1774)), apareciendo como primeras citas para la provincia: *Bithynia* (*Bithynia*) *tentaculata* (Linnaeus 1758), *Potamopyrgus antipodarum*, *Hydrobia* (*Hydrobia*) *acuta* (Draparnaud 1805), *Mercuria emiliana* (Paladilhe 1869), *Islamia minuta* (Draparnaud 1805), *Stagnicola palustris* (O.F. Müller 1774), *Radix* aff. *auricularia* (Linnaeus 1758), *Radix balthica* Linnaeus 1758, *Bulinus* (*Isidora*) *truncatus contortus* (Michaud 1829), *Planorbarius metidjensis* (Forbes 1838), *Ferrisia* (*Pettancylus*) *clessiniana* (Jickeli 1882), *Planorbis* (*Planorbis*) *carinatus* O.F. Müller 1774, *Gyraulus* (*Gyraulus*) *albus* (O.F. Müller 1774), *Hippeutis complanatus* (Linnaeus 1758), *Myosotella myosotis* (Draparnaud 1801), *Potomida littoralis* (Cuvier 1798), *Musculium* (*Musculium*) *lacustre* (O.F. Müller 1774), *Pisidium* (*Euglessa*) *casertanum* (Poli 1791), *Pisidium* (*Euglessa*) *personatum* Malm 1855 y *Pisidium* (*Cingulipisidium*) *milium* Held 1836. El número de especies por curso de agua ha oscilado entre 1 y 9 ($\bar{x}$= 2,6, sd= 1,5); la cuenca de los ríos Guadiana-Piedras es la más diversa (23 especies, H= 3,75), la menos diversa la del río Tinto (12 especies, H= 2,19).

DISCUSIÓN

Bithynia (*Bithynia*) *tentaculata* (Fig. 2, Cobertura (C)= 2,3%, Abundancia (A)= 1,3%) es una especie holártica (BOETERS, 1998), residente en Huelva en cauces de poca profundidad sobre sustratos rocosos o macrófitas de la cuenca del Guadiana; una concha vacía en la rivera de Nicoba, cuenca del río Tinto, atestigua su presencia en la zona, aunque el grado de erosión de la misma y su ausencia en el resto de estaciones de la cuenca parecen indicar que, probablemente, la especie no se encuentre actualmente en el entorno de dicho río.

Potamopyrgus antipodarum (Fig. 2, C= 0,7%, A= 0,8%) es una especie originaria de Nueva Zelanda (PONDER, 1988), aunque ANISTRATENKO (1997) sugiere que individuos de este género han aparecido, a comienzos del siglo XX, en depósitos cuaternarios de Lituania, pudiendo existir en la actualidad más de una especie en Europa. En la provincia se encuentra muy localizada en tramos de cabecera de la cuenca del río Odiel, asociada siempre a sustratos rocosos y macrófitas sumergidas entre 5 y 30 centímetros de profundidad, alcanzando densidades de más de 100 individuos por metro cuadrado.

Hydrobia (*Hydrobia*) *acuta* (Fig. 2, C= 1,5%, A= 0,8%) es una especie residente en las costas de Europa occidental en entornos litorales con salinidad superior al 10‰ (WILKE, ROLAN y DAVIS, 2000). En la provincia se ha encontrado en cristalizadores de salinas de extracción industrial situadas en marismas mareales del Paraje Natural Marismas del Odiel.

Peringia ulvae (Pennant 1777) (Figura 2, C= 0,7%, A= 0,4%) se distribuye a lo largo de las costas de Europa occidental, en el sedimento litoral, con un intervalo de salinidad de 4-33‰ (KERNEY, 1999). En la provincia se encuentra en marismas mareales con influencia continental en la desembocadura del río Piedras, entre sedimentos y sustratos rocosos.

Mercuria emiliana (Fig. 2, C= 2,3%, A= 1,3%) ha sido descrita en localidades de Granada, Alicante, Tarragona y Mallorca (BOETERS, 1988), recientemente ha sido encontrada en riveras de Almería (BAYO MONTORO, comunicación personal). En Huelva se encuentra en la cuenca baja del río Guadiana, en sustratos rocosos de cursos de agua dulce.

Islamia minuta (Fig. 2, C= 5,5%, A= 6,1%) es una especie abundante en cursos de agua leníticos o lóticos, en entornos calcícolas, de la cabecera de las cuencas de los ríos Guadiana, Odiel y Guadalquivir. Esta especie fue nominada originalmente como *Valvata globulina*, familia Valvatidae, posteriormente BINDER (1967a, 1967b) demuestra que la ornamentación

de su protoconcha es propia de la familia Hydrobiidae, habiendo sido ubicada dentro del género *Neohoratia* Schütt 1961 por BOETERS (1998), TURNER, KUIPER, THEW, BERNASCONI, RÜETSCHI, WÜTHRICH y GOSTELI (1998) y FALKNER, RIPKEN y FALKNER (2002), mientras que el comité del proyecto CLECOM (2002) la considera sinónima del género *Islamia* Radoman 1974, considerando como nombre válido para la especie el de *Islamia minuta*.

El género *Melanopsis* Férussac 1807 es muy polimórfico cariotípica (BARSIENE, TAPIA y BARSYTE, 1998) y conquiológicamente (GLAUBRECHT, 1996; PUJANTE, TAPIA y MARTÍNEZ, 1998). *Melanopsis praemorsa* (Fig. 2, C= 2,3%, A= 1,7%) se caracteriza por tener morfotipo liso sin quillas en la teloconcha (PUJANTE, comunicación personal), distribuyéndose en la Península Ibérica por Andalucía centro-occidental (GÓMEZ, MORENO, ROLÁN, ARAUJO y ÁLVAREZ, 2001); en la provincia de Huelva se encuentra únicamente en la cuenca del río Guadalquivir, en cursos de cabecera de la rivera de Huelva, en medios leníticos e incluso en acequias.

Actualmente los únicos criterios fiables para la correcta determinación de *Stagnicola palustris* (Fig. 2, C= 0,7%, A= 0,4%) se basan en el estudio morfológico del aparato reproductor y en el análisis de la sección terminal de la próstata (GLÖER y MEIER-BROOK, 2003), siendo la presente la primera confirmación de la presencia de la especie en España basada en el estudio de la anatomía de su aparato reproductor (MARTÍNEZ ORTÍ, comunicación personal). En la provincia de Huelva se encuentra en una sola localidad de cabecera de la cuenca del Odiel, en un entorno de intenso manejo humano del Parque Natural de la Sierra de Aracena y Picos de Aroche, en aguas leníticas y profundidad entre 20 y 100 cm, menos frecuentemente fuera del agua.

Galba truncatula (Fig. 2, C= 12,5%, A= 8,3%) es una especie holártica de carácter anfibio que se distribuye entre zonas de cabecera y marismas mareales (KERNEY, 1999). Se ha encontrado en todas las cuencas analizadas, siendo frecuente que aparezca asociada a rocas emergidas a 10-30 cm del borde del agua.

Radix aff. *auricularia* (Fig. 2, C= 2,3%, A= 1,7%) reside en aguas leníticas hasta con un 6‰ de salinidad (GIROD, BIANCHI y MARIANI, 1980), habiendo sido encontrada en la provincia en la subcuenca de la rivera de Huelva (cuenca del Guadalquivir) en sustratos rocosos de distintas superficies, y en entornos de marisma dulce de la cuenca del río Piedras en fondos de cauces con sustratos de limos y arcillas.

La sistemática de los Lymnaeidae europeos ha sido revisada recientemente desde el punto de vista molecular (BARGUES, VIGO, HORAK, DVORAK, PATZNER, POINTIER, JACKIEWICZ, MEIER-BROOK y MAS-COMA, 2001) y de prioridad de nomenclatura (FALKNER *ET AL.*, 2002), concluyendo en ambos casos que la especie *Radix peregra* (Müller 1774) es sinónima de *Radix balthica* (Linnaeus 1758). Esta es una especie eurícora que puede residir desde el crenon hasta en aguas mesohalinas con salinidad del 14‰ (GIROD *ET AL.*, 1980); en Huelva (Figura 2, C= 14,9%, A= 17,9%) aparece en todas las cuencas muestreadas, desde en aguas muy limpias hasta ligeramente eutrofizadas, incluso ha sido encontrada fuera del agua en fuentes y acequias.

Physella (*Costatella*) *acuta* (Draparnaud 1805) se distribuye en Norteamérica, Europa y África, siendo uno de los gasterópodos más abundantes de la Península Ibérica (VIDAL-ABARCA y SUÁREZ, 1985); es una especie de amplios requerimientos ecológicos que se encuentra indistintamente en cabeceras y desembocaduras, asociada a medios lóticos o leníticos desde 0 hasta 2 m de profundidad (MOUTHON y DUBOIS, 2001). Es la especie más ampliamente representada en la provincia de Huelva (Fig. 2, C= 61,4%, A= 67,9%), encontrándose en todas las cuencas en aguas limpias o muy eutrofizadas.

Bulinus truncatus contortus (Fig. 2, C= 2,3%, A= 1,7%) se distribuye a lo largo de la región mediterránea, Portugal y localidades del sudeste de Asia y África (BROWN, 1980); en Huelva se localiza en entornos lagunares de la cuenca del Odiel, encharcamientos someros temporales del Parque Nacional de Doñana y

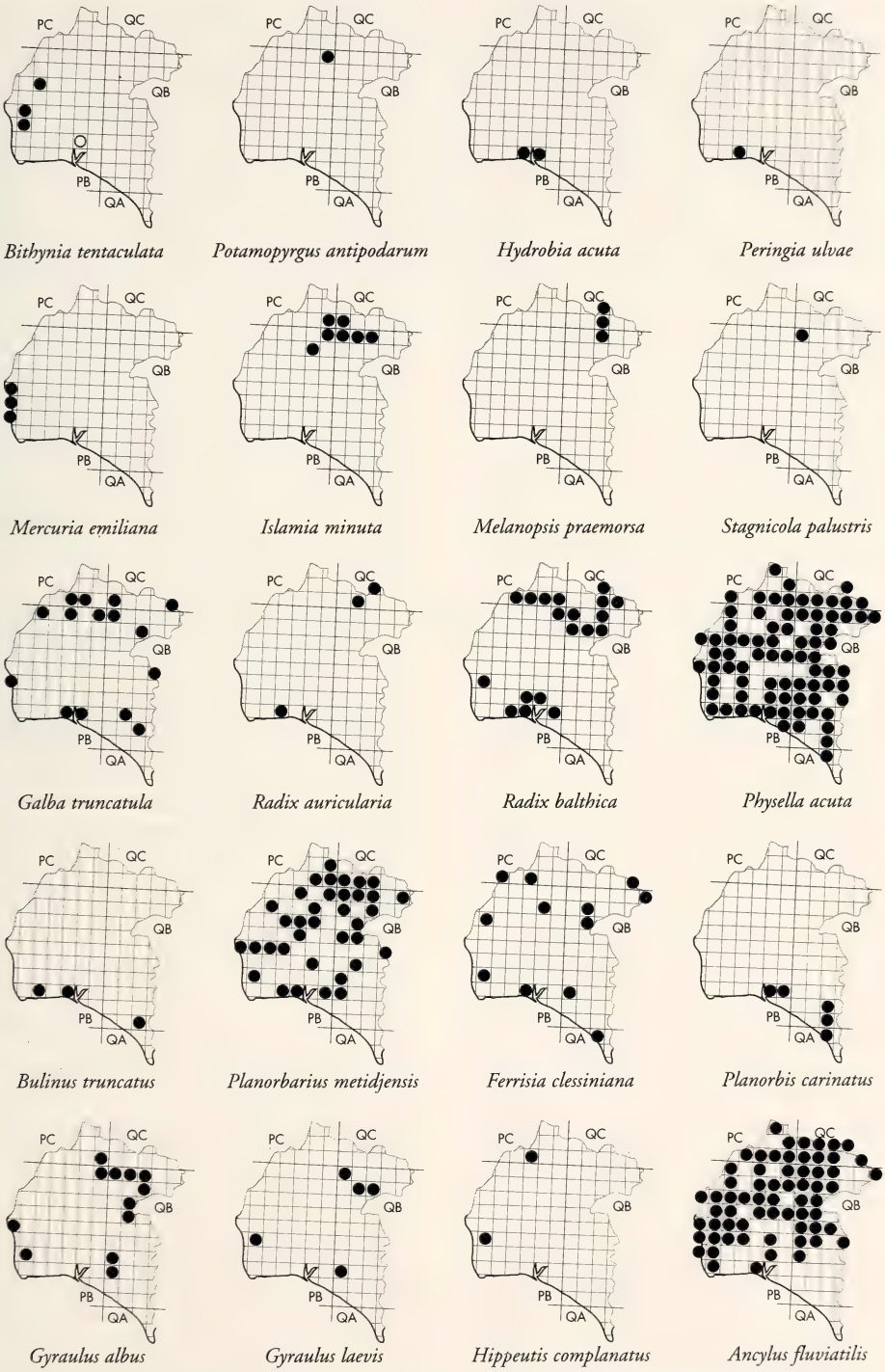

Figura 2. Mapas de distribución. Los círculos blancos indican la presencia de conchas o valvas vacías.

Figure 2. Distribution maps. White circles are empty shells or valves.

pequeños tributarios del río Piedras, siempre asociada a macrófitos o superficies cubiertas por películas de algas.

Planorbarius metidjensis (Fig. 2, C= 28,3%, A= 24,5%) se distribuye por la Península Ibérica y norte de África (VIDAL-ABARCA y SUÁREZ, 1985); es una especie tolerante a condiciones microambientales, lenitófila, muy dependiente del sustrato vegetal y residente en aguas de diferente dureza y salinidad (MEDEIROS y SIMÕES, 1987). En la provincia se encuentra en todas las cuencas analizadas, localizándose siempre en aguas leníticas asociada a sustratos rocosos de gran superficie y, en menor medida, sobre macrófitos.

La presencia de *Ferrisia clessiniana* (Fig. 2, C= 9,4%, A= 7,0%) fue descrita por primera vez en la Península Ibérica, como *Ferrisia wautieri* (Mirolli, 1960), por ALTABA, TRAVESET, BOGUÑÁ y BECH (1985); es una especie muy polimórfica, residente en aguas calmas o con flujo moderado (WAUTIER, 1977). Se ha encontrado en aguas leníticas de las cuencas de los ríos Guadiana, Odiel, Tinto y Guadalquivir, sobre vegetación o, más frecuentemente, sustratos rocosos de distinta superficie.

Planorbis carinatus (Fig. 2, C= 3,9%, A= 1,7%) se distribuye por Europa y oeste de Siberia (VIDAL-ABARCA y SUÁREZ, 1985). En Huelva se ha encontrado en humedales del entorno del Parque Nacional de Doñana y en las cuencas de los ríos Tinto y Guadalquivir, siempre asociado a macrófitos o sustratos inertes de pequeña superficie.

Las especies del género *Gyraulus* Charpentier 1837 que se encuentran en la provincia son de carácter lenitófilo y se asocian a limos y rocas de gran superficie. *Gyraulus albus* (Fig. 2, C= 9,4%, A= 6,1%) se encuentra en las cuencas de los ríos Guadiana, Odiel y Tinto desde cabecera hasta desembocadura; *Gyraulus laevis* (Alder 1838) (Fig. 2, C= 3,9%, A= 3,9%) en las cuencas de Guadiana, Odiel y Guadalquivir.

Hippeutis complanatus (Fig. 2, C= 1,5%, A= 0,8%) es una especie particularmente común en aguas eutrofizadas (COSTIL y CLEMENT, 1996). En Huelva se encuentra en entornos leníticos y, en la subcuenca del río Chanza, muy eutrofizados, sobre macrófitos o sustratos rocosos de la cuenca del Guadiana.

Ancylus fluviatilis O.F Müller 1774 (Fig. 2, C= 50,3%, A= 61,8%) es una especie paleártica muy difundida en la Península Ibérica (VIDAL-ABARCA y SUÁREZ, 1985), litófila y de marcado carácter reófilo (GELDIAY, 1956), que muestra un acusado polimorfismo en relación al carácter lótico o lenítico de los cursos de agua donde reside (ANGELIER, 2002). En la provincia de Huelva es la segunda especie en cobertura y abundancia, distribuyéndose desde cabecera hasta desembocadura en corrientes lóticas o leníticas de todas las cuencas analizadas; se ha encontrado una elevada correlación presencia-ausencia entre esta especie y *Physella acuta* (correlación por rangos de SPEARMAN, R=0,829, p= 0,0001).

Myosotella myosotis (Fig. 3, C= 0,7%, A= 0,8%) es una especie halófila atlántico-mediterránea presente en costas, marismas y salinas, que vive principalmente fuera del agua (KERNEY, 1999). En la provincia se encuentra en marismas mareales y desembocaduras de ríos, sobre vegetación emergida o en limos y barros de fondo o superficie de la cuenca del río Piedras.

Potomida littoralis (Fig. 3, C= 0,7%, A= 0,4%) es una especie con grandes problemas de conservación derivados de distribuciones muy localizadas y de competencia con especies introducidas (GÓMEZ *ET AL.*, 2001), habiendo sido propuesta por ello para su inclusión en los listados de especies protegidas por el Convenio de Berna (BOUCHET, FALKNER y SEDDON, 1999). Se encuentra en aguas leníticas sobre sedimentos finos de la cuenca del Guadiana.

Unio pictorum (Fig. 3, C= 5,5%, A= 5,2%) es una especie ampliamente distribuida en la región paleártica (ELLIS, 1978) y muy abundante en la Península Ibérica (VIDAL-ABARCA y SUÁREZ, 1985). Se han encontrado ejemplares vivos en las cuencas de los ríos Odiel y Guadiana, siempre sobre lechos de limos y arcillas y entre sustratos rocosos de pequeña superficie (< 10 cm de diámetro mayor), y

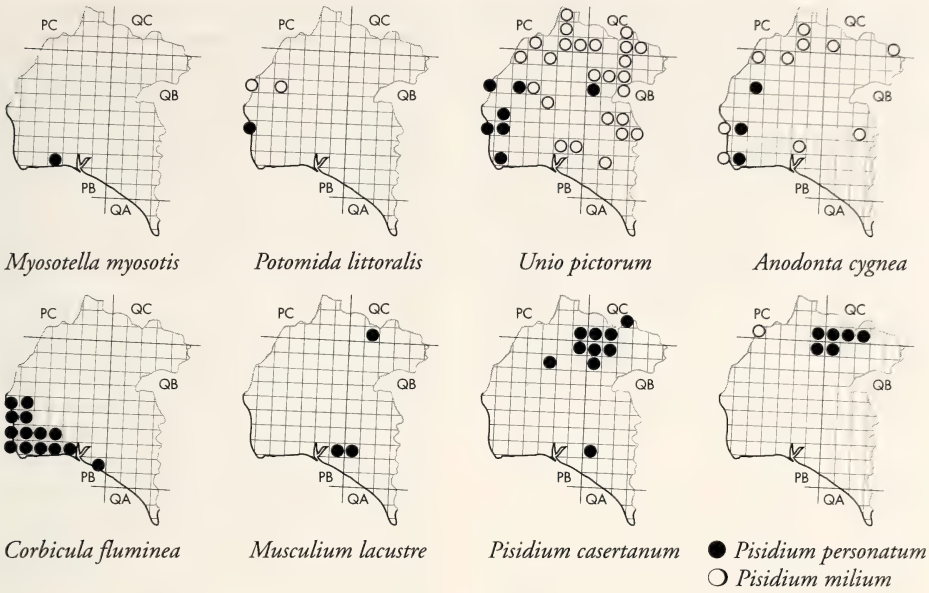

Figura 3. Mapas de distribución. Los círculos blancos indican la presencia de valvas vacías.
Figure 3. Distribution maps. White circles are empty shells or valves.

únicamente valvas vacías en la cuencas de los ríos Guadalquivir y Tinto.

El amplio conocimiento de la biología de *Anodonta cygnea* (Fig. 3, C= 2,3%, A= 1,7) en relación con su reproducción (GALHANO y FERREIRA, 1983), con su papel como bioacumulador de metales pesados (GUNDACKER, 2000) o con su particular polimorfismo en relación a la profundidad (MÜLLER y PATZNER, 1996), muestra a este bivalvo como una de las especies de moluscos dulceacuícolas más estudiada de nuestro entorno paleártico. Se han encontrado ejemplares vivos únicamente en la cuenca del río Guadiana, siempre enterrados en sedimentos finos alternados con sustratos rocosos de pequeña superficie.

La primera cita de *Corbicula fluminea* (Fig. 3, C= 11,0%, A= 3,0%) en la Península Ibérica es de MOUTHON (1981) en Portugal; posteriormente PÉREZ-QUINTERO (1990) la cita por primera vez en España, asociada a la cuenca del río Guadiana en la provincia de Huelva, y ARAUJO, MORENO y RAMOS (1993) amplían su distribución en el entorno europeo. Su enorme potencial biológico, dispersivo y de resistencia a las fluctuaciones del medio (ELDER y COLLINS, 1991) hacen de esta especie invasora una formidable competidora, desplazando y abocando a extinciones locales a la fauna autóctona, originando igualmente serios problemas en sistemas de conducción de aguas asociados a regadíos o consumo humano (STITES, BENKE y GUILLESPIE, 1995), como sucede en la zona regable del Chanza, Lepe, y en las conducciones de agua potable de la ciudad de Huelva (obs. pers.). Se encuentra en las cuencas de los ríos Guadiana y Piedras, en corrientes lentas y sustratos de sedimentos finos, alcanzando densidades superiores a 200 ind./m^2 en algunas zonas.

Musculium lacustre (Fig. 3, C= 2,3%, A= 1,3%) ha sido encontrada a escasa profundidad en corrientes lentas y entre sedimentos finos, con dos intervalos altitudinales bien diferenciados: 405 metros (en una acequia de la cuenca del Guadalquivir) y 88 metros (en un humedal cercano al Parque Nacional de Doñana).

De las tres especies del género *Pisidium* Pfeiffer 1821 encontradas en la provincia de Huelva, sólo *Pisidium casertanum* (Fig. 3, C= 7,8%, A= 8,7%) y *Pisidium persona-*

tum (Fig. 3, C= 4,7%, A= 3,5%) registran amplia distribución en la Península Ibérica, mientras que *Pisidium milium* (Fig. 3) se localiza en entornos concretos de la cuenca mediterránea (KUIPER, 1961; VIDAL-ABARCA y SUÁREZ, 1985). Todas ellas residen en aguas de flujo lento, entre sedimentos finos y a escasa profundidad (15-85 cm), *P. casertanum* entre 116-740 m de altitud, *P. personatum* entre 360-640 m, ambas en la cuenca de los ríos Guadiana, Odiel y Guadalquivir, y *P. milium*, de la que no se han encontrado individuos vivos sino solamente valvas, a 160 m de altitud en la cuenca del Guadiana.

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Anexo

Riveras muestreadas; en cada una de ellas aparece la referencia UTM de cada estación, la altura de la misma en metros (A) y las especies encontradas (*individuos muertos). Junto al nombre de la cuenca aparece la riqueza taxonómica de la misma, referenciada como (número de especies/géneros/familias), y el índice de diversidad de Shannon-Weaver H.
Sampled rivers, with UTM references, high (A) and species found (dead individuals). Between the name of the basin, values of taxonomic richness (number of species/genera/families) and diversity index (Shannon-Weaver H) are given.*

Bt: *Bithynia tentaculata*; Pa: *Potamopyrgus antipodarum*; Ha: *Hydrobia acuta*; Pu: *Peringia ulvae*; Me: *Mercuria emiliana*; Im: *Islamia minuta*; Mp: *Melanopsis praemorsa*; Sp: *Stagnicola palustris*; Gt: *Galba truncatula*; Ra: *Radix aff. auricularia*; Rb: *Radix balthica*; Pt: *Physella acuta*; Bc: *Bulinus truncatus contortus*; Pm: *Planorbarius metidjensis*; Fc: *Ferrisia clessiniana*; Pc: *Planorbis carinatus*; Ga: *Gyraulus albus*; Gl: *Gyraulus laevis*; Hc: *Hippeutis complanatus*; Af: *Ancylus fluviatilis*; Mm: *Myosotella myosotis*; Pl: *Potomida littoralis*; Up: *Unio pictorum*; Ac: *Anodonta cygnea*; Cf: *Corbicula fluminea*; Ml: *Musculium lacustre*; Ps: *Pisidium casertanum*; Pp: *Pisidium personatum*; Pi: *Pisidium milium*.

CUENCA DEL GUADALQUIVIR (19/16/8) H= 3,49

NOMBRE	UTM	A	ESPECIES	NOMBRE	UTM	A	ESPECIES
Arroyo Fuente del Rey	29SQB1798	740	Pm, Af, Ps	Arroyo Borbolluela	29SQC0704	430	Pt, Fc, Gl, Af, Up, Ac
Barranco Fuente el Castaño	29SQB1298	680	Pm, Af	Rivera de Cala	29SQC4702	430	Gt, Pt, Af
Arroyo de la Parrilla	29SQC2514	650	Rb, Ps	Rivera de Huelva	29SQC0803	420	Pt, Af, Up*, Ac*
Barranco las Huertas	29SQC2613	640	Rb, Af	Rivera de Montemayor	29SQC2009	405	Mp,Ra,Rb,Pt,Pm,Af,Up,Ml,Ps
Arroyo Guijarra	29SQB0999	620	Rb, Pm, Ga, Af	Barranco Fuente del Puerto	29SQB2098	400	Pm, Ga
Barranco del Lavadero	29SQC0801	620	Im, Rb, Pm, Ps	Rivera de Cala	29SQB5096	360	Pt, Fc, Af
Barranco Navahermosa	29SQC0401	620	Im, Gt, Rb, Pm, Af, Ps	Arroyo las Casetillas	29SQB2199	350	Im, Mp, Rb, Pt, Pm, Af
Barranco del Toro	29SQC0809	570	Pm, Af	Barranco Maygalanes	29SQB2989	350	Pm, Ga, Af
Barranco Fuente la Madrona	29SQB2196	570	Pm	Arroyo del Rey	29SQB2885	330	Rb, Pt, Gl, Af, Up*
Arroyo Guijarra	29SQC0901	560	Af	Rivera de Hierro	29SQB2895	270	Pm, Ga, Af
Barranco Valdelamadera	29SQC3009	535	Mp, Pt, Pm, Af	Rivera de Huelva	29SQB2895	260	Pt, Af, Up
Arroyo las Vegas	29SQC3010	530	Mp, Rb, Af, Up, Pp	Arroyo Alcarayón	29SQB2842	110	Pt, Up*, Ac*
Arroyo Carboneras	29SQB1599	520	Af	Arroyo de la Cerrada	29SQB1834	90	Pt
Barranco del Ingenio	29SQC0201	520	Pm, Ps	Arroyo de don Gil	29SPB9921	78	Pt
Charco de la Cuchara	29SQB4299	520	Pm	Arroyo Barbacena	29SQB3552	75	Gt, Pt, Pm
Arroyo Corterrangel	29SQC1101	485	Af, Pp	Arroyo del Avispero	29SQB0526	75	Pt, Fc, Gl
Rivera de Hinojales	29SQC1906	465	Pt	Arroyo de la Cañada	29SQB1522	63	Gt, Pt
Arroyo Castañuelo	29SQC1202	460	Ra, Rb, Pt, Af	Arroyo del Saltillo	29SQB1825	54	Pt
Rivera de Cala	29SQC4305	455	Pt, Fc, Gl, Af, Up, Ac	Arroyo de Tejada	29SQB3348	50	Pt, Af, Up*
Arroyo los Cocederos	29SQB2590	450	Gt, Pt, Pm, Af	Arroyo del Algarbe	29SQB3331	50	Pt
Barranco Descansadero	29SQB4497	450	Pt, Pm	Arroyo de Calancha	29SQB1627	40	Pt
Arroyo de Santa María	29SQB2024	35	Pt	Charca del Brezo	29SQA2196	2,1	Pt
Arroyo de la Rocina	29SQB0816	30	Pt	Caño las Gangas	29SQB2279	2,0	Pt
Arroyo de la Mayor	29SQB2818	20	Pt	Caño del Martinazo	29SQB1178	2,0	Pt
Arroyo de la Palmosa	29SQB2517	20	Pt	Veta del Martinazo	29SQB1683	2,0	Gt, Pt, Bc, Pc
Arroyo Madre del Avitor	29SPB9115	15	Pt, Cf	Laguna de Santa Olalla	29SQA2495	1,9	Pt, Fc
Arroyo de la Rocina	29SQB2412	8	Pt, Pc	Laguna del Taraje	29SQA2296	1,9	Pt
Laguna del Moral	29SQA2100	3,8	Pt	Charca de Wouter	29SQA2496	1,6	Pt
Laguna 12 del Martinazo	29SQB1868	3	Pt	Laguna Dulce	29SQA2395	1,5	Pt
Charco del Navazo del Toro	29SQA2299	2,9	Pt	Lucio del Bolín	29SQA2796	1,5	Pt
Charca del Raposo	29SQA2297	2,8	Pt	Charca Raya Pinar	29SQB2597	1,1	Pt
Laguna de Poli	29SQA2198	2,7	Pt	Laguna Fría	29SQA2397	0,7	Pt

CUENCA DEL TINTO (12/12/6) H= 2,19

NOMBRE	UTM	A	ESPECIES	NOMBRE	UTM	A	ESPECIES
Rivera de la Adelfilla	29SQB1983	400	Af	Arroyo de Pasadera	29SQB0130	83	Pt
Rivera de las Majadillas	29SQB1879	340	Pm, Ga, Af	Arroyo la Peñuela	29SQB0344	80	Pt, Af
Rivera de los Pinos	29SQB0664	290	Pm	Arroyo del Arzobispo	29SQB0938	70	Pt
Arroyo Tamujoso	29SQB1372	280	Pt, Af	Arroyo de Sapo Hondo	29SQB1140	65	Pt, Pm
Rivera del Jarrama	29SQB2175	270	Pt, Fc, Up*	Arroyo de la Grulla	29SPB9621	59	Rb, Pt, Pm, Pc, Ml
Arroyo el Barrancoso	29SQB0652	180	Pt, Af	Arroyo Clarina	29SQB1144	50	Pt
Rivera Cachán	29SQB1368	180	Af	Arroyo Giraldo	29SQB1643	45	Pt
Arroyo Bajohondo	29SQB0061	160	Pt	Arroyo Lavapies	29SQB0439	45	Pt, Af
Arroyo Gallego	29SQB1866	160	Pt, Pm, Ga	Arroyo Candón	29SQB0040	35	Pt, Af
Rivera Casa de Valverde	29SQB1057	130	Pt, Af, Up	Rivera de Nicoba	29SPB8939	35	Pt, Up*
Arroyo del Pinar Serrano	29SQB0127	116	Ga, Ml, Ps	Arroyo del Puerco	29SPB8439	25	Af
Arroyo Tamujoso	29SQB1851	115	Pt, Af	Arroyo Lavapiés	29SQB0437	25	Pt
Río Corumbel	29SQB2450	115	Pt, Up*	Rivera de Nicoba	29SPB8737	25	Up*
Arroyo Gallinero	29SQB1851	110	Pt	Arroyo del Puerco	29SPB8439	22	Af, Up*
Rivera del Hornueco	29SQB1663	110	Pm, Af	Arroyo del Moro	29SQB0336	15	Pm, Ga
Arroyo de Trigueros	29SPB9444	105	Pt	Arroyo Candón	29SPB9935	9	Pt
Arroyo de la Fuentidueña	29SQB2045	90	Pt	Arroyo Candón	29SPB9834	7	Pt, Up*
Arroyo de Pozo Ancho	29SPB9244	65	Pt	Estero Domingo Rubio	29SPB8820	7	Pt, Pc
Arroyo de la Bárcena	29SPB9244	85	Pt	Arroyo de Freire	29SPB8427	4	Gt, Pt
Arroyo el Guijillo	29SQB0243	85	Pt	Rivera de Nicoba	29SPB8432	4	Bt*, Pt, Ac*

CUENCA DEL ODIEL (15/13/5) H= 3,04

NOMBRE	UTM	A	ESPECIES	NOMBRE	UTM	A	ESPECIES
Rivera de Alájar	29SQB0594	700	Im, Sp, Pt	Rivera Escalada	29SPB9790	320	Rb, Pm, Af
Rivera de Almonaster	29SPB9594	640	Pa, Im, Rb, Af, Pp	Rivera la Pelada	29SPB8386	320	Pt, Pm, Fc, Af
Barranco la Umbría	29SQB2393	600	Af	Río Odiel	29SQB1686	310	Rb, Pt, Fc, Af, Up
Arroyo de Marimateos	29SQB1895	580	Pt, Pm, Ga, Af	Rivera del Vaho	29SPB0087	281	Pm, Af
Barranco de la Lana	29SPB9794	580	Gt, Rb, Pm, Af, Ps	Rivera de Santa Eulalia	29SQB0587	280	Rb, Af, Up*
Barranco los Casares	29SQB0194	580	Im, Gt, Rb, Pt, Pm, Gl, Af	Rivera Escalada	29SPB9887	275	Pt, Af
Rivera de las Molinillas	29SQB1295	580	Im, Af	Rivera del Villar	29SQB0073	235	Pt, Af, Up*
Barranco del Acebuche	29SPB9295	560	Gt, Rb, Pt, Pm, Af	Arroyo de la Burrilla	29SPB5654	210	Pt, Pm, Af
Rivera de Santa Ana	29SQB0293	540	Rb, Af, Ps	Rivera Olivargas	29SPB9084	210	Pt, Af
Barranco el Cabezuelo	29SQB0494	500	Im, Rb, Af, Ps	Barranco la Fresnera	29SPB7583	200	Im, Af, Ps
Barranco de Calabazares	29SPB9793	460	Rb, Af, Ps	Barranco del Tamujoso	29SPB8576	185	Pt, Af
Arroyo Plamencia	29SQB0994	440	Rb, Pm, Gl, Af	Barranco de los Pinos	29SPB8179	180	Pt, Pm, Af
Barranco los Madroñeros	29SQB0693	440	Af	Barranco de los Ovejeros	29SPB7375	160	Pt, Pm, Af
Rivera de Almonaster	29SPB9392	440	Pa, Im, Rb, Af	Arroyo Galaperosa	29SPB8562	150	Pt, Af
Rivera de las Molinillas	29SQB0993	380	Im, Af	Barranco de la Sepultura	29SPB7174	140	Pt, Af
Barranco la Cabra	29SPB9991	360	Rb, Pm, Af, Ps, Pp	Barranco de Juré	29SPB6857	120	Pt, Pm, Af
Rivera del Villar	29SQB0774	360	Pt, Af	Arroyo de las Multas	29SPB6549	100	Pt, Af
Río Vanegas	29SQB1687	330	Pt, Fc, Gl, Af	Arroyo Carrasco	29SPB9260	90	Pt, Af
Rivera de Santa Ana	29SQB0589	330	Gt, Rb, Af, Ps	Arroyo Monte de la Osa	29SPB7465	85	Pt, Af, Up*
Arroyo del Tejarejo	29SQB1586	320	Pt, Fc, Af	Arroyo Chapinero	29SPB7567	80	Pt, Pm, Af
Barranco el Moro	29SPB9091	320	Rb, Af	Arroyo del Encinar	29SPB8242	35	Pt, Pm, Af

CUENCA DEL GUADIANA-PIEDRAS (23/19/9) H= 3,75

NOMBRE	UTM	A	ESPECIES	NOMBRE	UTM	A	ESPECIES
Barranco la Higueruela	29SPB9898	600	Af, Ps	Rivera del Chanza	29SPC8103	270	Pt, Af
Rivera de Jabugo	29SQB0098	600	Im, Gt, Rb, Pm, Af, Ps, Pp	Arroyo Arochete	29SPC8005	260	Pt, Fc, Af, Up*
Arroyo Sillo de Cumbres	29SQC1015	580	Af	Río Múrtigas	29SPC9115	260	Pt, Af
Rivera de Jabugo	29SQB0099	580	Im, Rb, Af	Rivera de los Ciries	29SPC7596	260	Pt, Af
Barranco el Buho	29SPC9303	540	Im, Rb, Pm, Af	Rivera Peramora	29SPC7597	260	Gt, Af, Pt, Up*, Ac*
Barranco la Urraca	29SQB0299	540	Rb, Pm, Af, Ps, Pp	Rivera del Chanza	29SPC7806	255	Pt, Fc, Af
Arroyo Gargallones	29SPC8801	480	Rb, Af	Arroyo Sillo	29SPC8818	240	Pt, Up*, Ac*
Barranco el Puerto	29SPB9597	480	Rb, Af, Ps, Pp	Rivera Piernaseca	29SPB5790	240	Pt, Af

Arroyo del Cavá	29SPC8625	400	Pt, Af	Arroyo Valquemado	29SPC8122	220	Af
Rivera Caliente	29SPC9604	400	Af, Up*, Ps, Pp	Río Múrtigas	29SPC8222	220	Pt, Af, Up*
Río Múrtigas	29SPC9705	380	Af, Ps	Rivera Charcolino	29SPB6978	206	Pt, Pm, Af
Barranco las Murtiguillas	29SQC0006	360	Pt, Af	Rivera del Cañuelo	29SPB6778	205	Pt, Af, Up*
Barranco Riofrío	29SPC9911	360	Pt, Af	Arroyo las Cañas	29SPC5804	190	Pt, Fc
Barranco Menjuana	29SQC9910	350	Pt, Pm, Ga, Af	Arroyo Petaquera	29SPB4951	180	Pt, Af
Barranco la Extremedera	29SQC9809	320	Rb, Pt, Pm, Ga, Af	Rivera del Chanza	29SPC6304	180	Rb, Af, Up*
Barranco Riofrio	29SPC9809	310	Pt, Af, Up*	Rivera del Malagoncillo	29SPB6472	180	Pt, Af
Barranco Valdesotello	29SPC7808	285	Pt, Af	Arroyo de Agualobos	29SPB4776	175	Pt, Af, Up*
Barranco de la Villa	29SPC7902	280	Gt, Rb, Pt, Pm, Hc, Af	Arroyo Albahacar de Allá	29SPB5180	170	Pt, Af
Barranco los Cubos	29SPC8203	280	Pt, Af	Rivera de Malagón	29SPB5173	170	Bt, Pt, Af, Pl*, Up, Ac
Rivera del Chanza	29SPC8202	275	Gt, Rb, Pt, Pm, Af, Up*, Ac*	Arroyo de los Arroyillos	29SPB5565	165	Pt, Af
Barranco de Monteviejo	29SPB5482	165	Pt, Pm, Af	Rivera de la Ronchona	29SPB3550	35	Cf, Pt
Barranco la Buharda	29SPC5503	160	Pt, Pi*	Arroyo del Contrapeso	29SPB3354	35	Af
Rivera de la Espada	29SPB5455	160	Pt, Af	Arroyo Grande	29SPB4430	30	Pt, Af, Up*, Ac*
Rivera del Aserrador	29SPB5694	160	Gt, Af, Up*	Arroyo de la Gitana	29SPB4726	25	Pm, Af
Arroyo del Colmenar	29SPB4944	150	Rb, Pt, Af	Barranco de la Chacera	29SPB3936	20	Me, Af
Barranco del Fraile	29SPB4779	150	Pt, Af	Arroyo Tariquejo	29SPB6129	15	Pu
Barranco el Arroyo	29SPB4776	150	Pt, Fc, Af	Rivera Grande	29SPB3747	15	Me, Gt, Af, Pl, Up, Ac, Cf
Rivera de Calaboza	29SPB5898	140	Af, Up*, Ac*	Río Piedras	29SPB6128	10	Mm
Barranco Redondillo	29SPB4261	120	Pt, Af	Arroyo del Pilar	29SPB6428	8	Ra, Rb, Pt, Pm
Rivera del Chanza	29SPB3979	95	Pt, Af, Pl*, Up	Arroyo Grande	29SPB4028	8	Pt, Fc, Af, Up, Ac, Cf
Rivera Matavacas	29SPB4248	95	Pt, Af	Arroyo de la Vera	29SPB5720	7	Pt
Arroyo del Alamillo	29SPB4632	90	Pt, Pm, Ga, Af	Arroyo Puentezuelo	29SPB6227	7	Ra
Rivera Agua de Miel	29SPB5774	120	Pt, Af	Marismas del Piedras	29SPB6220	0	Mm
Arroyo de la Poricona	29SPB4635	110	Pm, Ga, Af	Canal del Piedras	-	-	Rb, Cf
Rivera de la Ronchona	29SPB3853	90	Pt, Pm, Af	Arroyo del Prado	29SPB5220	5	Pt, Bc
Rivera de la Golondrina	29SPB4051	85	Bt, Pt, Pm, Ga, Af, Up, Cf	Barranco Huerto Torres	29SPB3650	5	Me, Af

Fatty acids of Antarctic gastropods: distribution and comparison with Mediterranean species

Ácidos grasos en gasterópodos antárticos: distribución y comparación con especies mediterráneas

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ABSTRACT

Fatty acids of three different lipid pools: free fatty acids (FFA), storage lipids (triglycerides and wax esters, SL) and phospholipids (PL) of mantle and viscera of Antarctic gastropods were analyzed and compared to species from the Mediterranean Sea. We analyzed specimens of the Antarctic species: *Bathydoris clavigera* Thiele, 1912, *Tritonia challengeriana* Bergh, 1884 and *Marseniopsis mollis* (Smith, 1902), and the Mediterranean species: *Hypselodoris picta* (Schultz, 1836) and *Dendrodoris limbata* (Cuvier, 1804). Fatty acid composition was very different between viscera and mantle of the same individuals, and the amounts of polyunsaturated fatty acids were significantly higher in the mantle. There were higher levels of polyunsaturated fatty acids in mantle phospholipids of Antarctic molluscs than in Mediterranean molluscs. Arachidonic and eicosapentaenoic acids were the dominant species of phospholipids in Antarctic molluscs, whereas octadecaenoic acid was the most abundant species in the phospholipid pools of Mediterranean animals. A comparison of the SFA/PUFA (saturated vs. polyunsaturated fatty acids) and MUFA/PUFA (monounsaturated vs. polyunsaturated fatty acids) indexes in SL and PL of Antarctic and Mediterranean specimens showed statistically significant differences among them, thus suggesting a relationship with environmental temperature.

RESUMEN

Se analizan los ácidos grasos de tres tipos de lípidos, ácidos grasos libres (FFA), lípidos de reserva (triglicéridos y ésteres de ceras, SL) y fosfolípidos (PL), en el manto y las vísceras de gasterópodos antarcticos, y se comparan con especies mediterráneas. Las especies estudiadas fueron las antarcticas *Bathydoris clavigera* Thiele, 1912, *Tritonia challengeriana* Bergh, 1884 y *Marseniopsis mollis* (Smith, 1902), y las mediterráneas *Hypselodoris picta* (Schultz, 1836) y *Dendrodoris limbata* (Cuvier, 1804). La composición de ácidos grasos difirió mucho entre vísceras y manto de algunos especímenes, y la cantidad de acidos grasos poliinsaturados fue significativamente más elevada en el manto. Se encontraron mayores niveles de éstos últimos en el manto de las especies antarcticas que en el de las mediterráneas. Los ácidos araquidónico y eicosapentanoico fueron los dominantes en los PL de las especies antarcticas, en los PL de las especies mediterráneas lo fue el ácido

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octadecanoico. La comparación entre los índices SFA/PUFA (ácidos grasos saturados vs. poliinsaturados) y MUFA/PUFA (ácidos grasos monoinsaturados vs. poliinsaturados) de los SL y PL de los dos grupos de especies mostró diferencias estadísticamente significativas entre ellos, lo que sugiere una relación con la temperatura ambiental.

KEY WORDS: fatty acids, Antarctic, gastropods, opisthobranch molluscs, chemical ecology.
PALABRAS CLAVE: ácidos grasos. Antártica, gasterópodos, moluscos opistobranquios, ecología química.

INTRODUCTION

Fatty acids in invertebrates are known to have broad biological roles, including lipid energy reserves, components of cellular structures such as biomembranes, and regulation of biosynthesis of eicosanoids, among others. Environmental conditions, such as diet or temperature, are closely related to lipid metabolism and may modulate the activities of the membrane (VOOGT, 1983; CULLIS AND HOPE, 1991; URICH, 1994; NELSON, LEIGHTON, PHLEGER AND NICHOLS, 2002). In fact, the physical properties of cell membranes are affected by even minor variations in the proportions of phospholipids, glycolipids, sterols and fatty acids (CULLIS AND HOPE, 1991; URICH, 1994). Also, it is well known that poikilotherms alter their membrane lipid composition in response to varying environmental temperature (URICH, 1994; MOON, HIGASHI, ZOLTAN AND MURATA, 1995; CHAKKODABYLU AND THOMPSON, 1984; DEY, BUDA, WIIK, HALVER AND FARKAS, 1993; HALL, THOMPSON AND PARRISH, 2000; FARKAS, FODOR, KITAJKA AND HALVER, 2001). The most common changes involve re-tailoring of phospholipid heads, sterol content of membranes and unsaturation of phospholipid fatty acids (URICH, 1994; CHAKKODABYLU AND THOMPSON, 1984; DEY *ET AL.*, 1993). One rational explanation of these variations is that cells compensate the temperature decrease with the increase of the membrane fluidity (*e.g.* the content of unsaturated fatty acids in cell membranes becomes higher at lower temperatures). For marine organisms, the temperature-dependent composition of membrane lipids has been reported in some bacteria (ROTERT, TOSTE AND STEIER, 1993; SAKAMOTO, HIGASHI, MURATA AND BRYANT, 1997; QUOC AND DUBACQ, 1997; RUSSELL, 1998; RUSSELL AND NICHOLS, 1999), unicellular algae (SATO, MURATA, MIURA AND UETA, 1979; THOMPSON, GUO, HARRISON AND WHYTE, 1992; LEHMAL, 1999), cnidarians (e.g. CARBALLEIRA, MIRANDA AND RODRÍGUEZ, 2002), molluscs (KATTNER, HAGEN, GRAEVE AND ALBERS, 1998; GILLIS AND BALLANTYNE, 1999; FREITES, LABARTA AND FERNÁNDEZ-REIRIZ, 2002), and fish (HAZEL, 1984; DEY *ET AL.*, 1993).

Gastropods are one of the most diverse animal groups, both in form, behavior and habitats (PONDER AND LINDBERG, 1997), and their evolutionary success could be related, among others, to their extraordinary ability to become adapted to different environments. This makes these animals particularly suitable for investigating the temperature effect on the cellular homeostasis. Gastropods have often been chemically studied for their ability to produce secondary metabolites with ecological significance or potential use as drugs or pharmacological tools (IRELAND, COPP, FOSTER, MCDONALD, RADISKY AND SWERSEY, 1993; ÁVILA, 1995; SHU, 1998), but surprisingly, they have received little attention regarding the effect of the environmental conditions on their lipid levels (URICH, 1994; KATTNER *ET AL.*, 1998; ISAY AND BUSAROVA, 1984). Actually, there are many descriptive studies on the primary lipids of prosobranchs (see VOOGT, 1983), but there have been almost no investigations on opisthobranchs. In fact, only some very early studies are reported on *Aplysia kurodai*

Table I. Data on the specimens studied quantitatively.
Tabla I. Datos de los especímenes estudiados cuantitativamente.

Species	No. of specimens analyzed	Depth (m)	Geographic area	Size (cm)	Wet (w) or Dry (d) weight (g)
Bathydoris clavigera	1	462	Weddell Sea	9.5	45 w
Tritonia challengeriana	1	446	Weddell Sea	5	3.5 w
Marseniopsis mollis	1	227	Weddell Sea	4.5	22.6 w
Hypselodoris picta	3	2-12	Mediterranean Sea	7.5-10	1.8-2.8 d
Dendrodoris limbata	2	2-12	Mediterranean Sea	5-8	0.8-1.2 d

(Baba, 1937) (TANAKA AND TOYAMA, 1959), *Aplysia fasciata* Poiret, 1789 and *Pleurobranchaea meckeli* Meckel in Leue, 1813 (TIBALDI, 1966), and more recently on the pteropod *Clione limacina* (Phipps, 1744) (KATTNER *ET AL.*, 1998).

During our ongoing research on natural products of marine invertebrates, we repeatedly observed high contents of fatty acids in extracts of Antarctic organisms, mainly opisthobranch molluscs and sponges. Therefore, we decided to further investigate this fact by carrying out a comparative study on the fatty acid content in Antarctic and Mediterranean gastropods. We selected two opisthobranch species and a single prosobranch species from Antarctica, and two Mediterranean opisthobranch species. We report here the composition and tissue distribution of their fatty acids in three different lipid pools: phospholipids (PL), storage lipids (SL, i.e. triglycerides and wax esters), and free fatty acids (FFA).

MATERIALS AND METHODS

Materials

The species studied here were the opisthobranchs *Bathydoris clavigera* Thiele, 1912 and *Tritonia challengeriana* Bergh, 1884 and the prosobranch *Marseniopsis mollis* (Smith, 1902), from Antarctica, and the opisthobranchs *Hypselodoris picta* (Schultz, 1836) and *Dendrodoris limbata* (Cuvier, 1804) from the Mediterranean (Table I). The species *T. challengeriana* had been named *Marionia cucullata* (Couthouy *in* Gould, 1852) in the past, but its taxonomy has been recently revised by MUNIAÍN AND SCHRÖDL (1999). Mediterranean species were selected due to our previous knowledge of their chemical ecology and their availability, while Antarctic species were selected because of their availability and their size (which should be large enough to allow for chemical analysis).

Preliminary qualitative studies were carried out with several specimens of these and other species not reported here, in order to test and improve the chemical methodology. Antarctic specimens used in this quantitative study were collected during the German expedition ANT XIII/3 (EASIZ I) to the Eastern Weddell Sea in January 1996 (Table I) (ARNTZ AND GUTT, 1997). The Mediterranean specimens were collected by scuba-diving in the Gulf of Naples (Italy) in June 1996 (Table I). All biological samples were immediately frozen and kept at –30°C until the chemical analyses were performed.

Dissection and extraction of molluscs

The specimens were carefully dissected in order to separate mantle and viscera. Since only a limited number of the Antarctic specimens was available, we used sub-samples from the animals in order to have pseudo-replicates. We analyzed two sub-samples for *T. challengeriana* and three for the other two Antarctic species, and two sub-samples for each type of tissue analyzed. Each body section was extracted separately

Table II. Content (µg per mg of total lipid extract) of SL, PL and FFA in mantle and viscera of the studied gastropods.
Tabla II. Contenido (µg per mg del extracto lipídico total) de SL, PL y FFA en manto y vísceras de los gasterópodos estudiados.

	SL		PL		FFA	
	Mantle	Viscera	Mantle	Viscera	Mantle	Viscera
Hypselodoris picta	71.0	322.5	14.6	31.0	44.4	75.3
Dendrodoris limbata	16.1	15.1	90.3	119.3	80.6	47.0
Marseniopsis mollis	344.8	58.5	293.1	265.4	193.1	61.2
Bathydoris clavigera	208.3	146.5	8.0	338.4	237.5	77.5
Tritonia challengeriana	242.8	242.0	158.5	228.5	250.0	35.7

by following the method of Bligh and Dyer (Hamilton, Hamilton and Sewell, 1992). The sample (ca. 8.5 mg) was homogenized by blending with a mixture of $CHCl_3$ (8.5 ml) and MeOH (17 ml) for two minutes. Then 8.5 ml of $CHCl_3$ were added to the solution and blended for 30 sec. more. Distilled water (8.5 ml) was added to the solution and the mixture was blended again for 30 sec. The suspension was filtered through paper on a Buchner funnel and the filtrate was recovered. The residue was transferred into the blender and the extraction was repeated. The $CHCl_3$ layer of the combined filtrates was separated and dried at reduced pressure to give a lipid extract containing glycerides, fatty acids, sterols and phospholipids.

Fractionation of the lipid extracts

The $CHCl_3$ soluble fractions were separated by SiO2 column (typically 70 mg silica *per* 1 mg of extract). Briefly, the lipid extract was solved in petroleum ether (typically 50 µl *per* 1 mg of extract) and loaded onto a column. Then 0.1 ml of 37% aqueous ammonia were added to the column and elution started with petroleum ether/diethyl ether (95:5 v/v) to give triglycerides, wax esters and sterols. Then glacial acetic acid (0.1 ml) was added to the column and the elution was completed by diethyl ether and a mixture of chloroform/methanol/water (60:40:2 v/v), to give free fatty acids and polar glycerides, respectively. The results of the separation by the SiO_2 column were checked by TLC for accuracy. In order to be further analyzed, the samples were transformed into fatty acid methyl esters (FAMEs).

Preparation of FAMEs

Fractions containing free fatty acids were concentrated at reduced pressure to a volume of 0.5 ml and reacted in open vials with a saturated solution of CH_2N_2 in Et_2O (0.5 ml). The reaction was performed for 30 min at room temperature. The excess of CH_2N_2 was removed by bubbling a stream of nitrogen. Solutions were concentrated under nitrogen flow to ca. 0.3 ml and analysed by GC-MS under the analytical conditions reported below.

Phospholipids and storage lipids (triglycerides and wax esters) were converted to FAMEs by a base catalyzed transesterification with Na_2CO_3 in dry methanol. Briefly, lipid samples were transferred to graduate screw-top vials and treated with 1.5 ml of saturated sodium carbonate in dry methanol. The reaction solution was heated at 40°C for 2 h, cooled at room temperature, transferred to a separating funnel and extracted with 5 ml diethyl ether and 8 ml of brine. The upper phase was removed and the extraction was repeated three times. The organic layers were combined, reduced to small volume (ca. 1 ml) and analyzed by GC-MS under the conditions reported below. We did not separate triglycerids

Table III. Relative amounts (mean % w/w ± SD) of the main fatty acids identified in mantle sections of Antarctic molluscs. SL: storage lipids (triglycerids and wax esters). PL: phospholipids. FFA: free fatty acids. nr: below the measurement limit. -: this sample could not be analyzed.
Tabla III. Cantidad relativa (porcentaje medio del peso húmedo ± SD) de los principales ácidos grasos identificados en las secciones del manto de moluscos Antárticos. SL: lípidos de almacenamiento (triglicéridos y ésteres de ceras). PL: fosfolípidos. FFA: ácidos grasos libres. nr: por debajo del límite de resolución. -: muestra no analizada.

	Marseniopsis mollis			*Bathydoris clavigera*			*Tritonia challengeriana*		
	SL	PL	FFA	SL	PL	FFA	SL	PL	FFA
16:0	28.8±7.2	16.2±5.5	5.0±1.9	-	14.7±3.1	10.7±3.3	1.6±1.1	nr	1.3±0.4
17:0	8.5±3.7	4.8±2.8	1.1±0.7	-	1.6±0.5	1.5±0.7	5.0±2.2	0.9±0.6	0.6±0.2
18:0	22.1±6.7	6.1±2.1	3.3±1.4	-	9.1±3.1	6.4±2.4	8.8±2.4	8.6±3.2	7.6±2.8
16:1 ω7	4.5±2.1	0.6±0.2	1.2±0.5	-	5.0±2.8	3.8±1.0	28.5±7.7	13.9±3.6	14.8±4.4
18:1 ω7/ ω 9	14.5±4.4	7.1±3.0	5.3±1.4	-	6.6±2.8	7.5±2.0	13.0±5.1	5.6±2.1	9.7±3.4
20:1	11.0±3.9	14.1±5.6	12.2±6.5	-	9.1±2.8	10.4±4.1	10.4±5.1	10.2±2.8	9.5±3.6
18:2 ω6	nr	nr	nr	-	3.6±1.6	1.2±0.7	nr	nr	1.1±0.4
20:4 ω6	1.0±0.4	5.6±1.8	26.6±3.1	-	13.8±4.1	22.2±6.3	20.7±7.2	43.8±7.9	40.9±5.4
20:5 ω3	2.8±1.3	29.2±8.6	15.9±4.9	-	11.6±4.2	11.1±4.3	2.7±1.4	4.8±1.2	5.5±1.5
22:5 ω6	nr	4.9±1.5	5.5±2.1	-	10.1±3.4	6.7±3.2	0.7±0.4	6.5±3.3	0.7±0.2
22:6 ω3	1.4±0.8	6.5±2.4	19.7±3.4	-	8.8±2.6	15.3±6.4	1.2±0.3	3.0±1.6	3.1±1.7

from wax esters, since this was not the aim of this study.

GC-MS analysis

Analysis of FAMEs was carried out on a Fisons MD800 Mass Spectrometer coupled to a Fisons GC8000 Chromatograph equipped with a J&W Scientific DB5-MS column (30 m x 0.25 mm x 0.25 µm). Helium was used as carrier gas at a flow rate of 1 ml/min. Each sample (1 µl) was injected in split mode (1:20). The oven temperature was programmed initially at 100°C for 3 min, and then increased to 300°C at 3°C/min; the injector and the transfer line temperatures were 260°C and 240°C, respectively. Mass spectra were recorded in continuous scan mode from 50 to 450 u.m.a. with an ionization current of 70 eV; the source temperature was set at 200°C. FAMEs were identified by both retention time, previously determined on a standard mixture with alkyl chains from C-12 to C-24, and by library-assisted interpretation of mass-spectra. Percentages were measured by analysis of the peak areas in the chromatogram, by using HP G1034C Chemstations software. The results were expressed as relative percentages (% w/w) of the total fatty acid content, and were compared by using t-tests to determine statistically significant differences.

RESULTS

The total lipid content in the viscera was similar in all the studied animals, with means of 28.0±2.4 mg/g of wet tissue in Antarctic samples and 25.2±3.3 mg/g in Mediterranean samples. In the mantle, the total lipid content was consistently higher in the Mediterranean molluscs (15.8±3.1 mg/g of wet tissue) respect to the Antarctic ones (3.2±0.4 mg/g of wet tissue). Three different lipid pools: free fatty acids (FFA), storage lipids (SL) and phospholipids (PL) were considered for each body part (Table II). The fatty acid composition of FFA, SL and PL was determined from mantle and viscera, and it is reported here for the Antarctic molluscs (Tables III and IV). Although fatty acid composition of FFA, SL and PL was not very different, the relative percentages varied

Table IV. Relative amounts (mean % w/w ± SD) of the main fatty acids identified in the viscera of Antarctic molluscs. SL: storage lipids (triglycerids and wax esters). PL: phospholipids. FFA: free fatty acids. nr: below the measurement limit.

Tabla IV. Cantidad relativa (porcentaje medio del peso húmedo ± SD) de los principales ácidos grasos identificados en las vísceras de moluscos Antárticos. SL: lípidos de almacenamiento (triglicéridos y ésteres de ceras). PL: fosfolípidos. FFA: ácidos grasos libres. nr: por debajo del límite de resolución.

	Marseniopsis mollis			*Bathydoris clavigera*			*Tritonia challengeriana*		
	SL	PL	FFA	SL	PL	FFA	SL	PL	FFA
16:0	5.2±1.4	4.5±1.8	2.2±1.2	2.9±2.0	4.3±0.7	2.5±0.6	16.7±3.7	9.5±2.1	6.9±1.9
17:0	5.3±1.4	8.7±0.6	0.4±0.1	0.7±0.2	1.8±0.6	0.5±0.1	7.3±1.5	5.5±1.0	3.1±0.9
18:0	12.4±0.3	29.6±0.6	3.6±1.6	9.1±0.4	14.2±1.8	4.3±0.9	5.9±1.1	9.1±2.2	3.3±2.2
16:1 ω7	4.7±0.2	4.5±1.3	1.1±0.7	3.3±0.4	2.6±1.0	6.9±1.4	30.3±5.5	12.5±3.6	28.4±4.0
18:1 ω7 /ω 9	13.5±1.0	17.9±0.9	18.7±1.0	18.3±0.8	12.0±1.1	15.3±1.4	23.4±2.7	11.8±1.4	15.1±1.6
20:1	24.2±2.2	17.2±3.2	13.3±0.8	15.5±1.6	18.7±1.4	16.2±0.6	2.3±0.9	4.0±1.9	0.7±0.4
18:2 ω6	4.0±0.9	5.4±1.1	3.0±0.8	5.3±0.5	3.8±0.3	1.1±0.5	8.9±2.3	11.8±3.0	18.2±3.7
20:4 ω6	6.0±0.9	1.8±0.9	7.9±1.3	18.1±1.2	15.9±0.9	19.3±1.0	nr	16.9±1.8	10.3±1.5
20:5 ω3	9.6±1.4	4.6±0.6	20.2±2.1	10.8±0.9	8.7± 0.9	11.4±0.8	nr	6.9±2.1	6.6±1.1
22:5 ω6	4.0±1.5	nr	7.2±0.4	3.5±0.9	5.0±0.4	5.1±1.1	nr	1.6±0.3	1.5±0.6
22:6 ω3	5.3±0.2	0.9±0.3	18.1±0.3	8.2±1.0	7.5±0.3	12.5±1.4	nr	5.0±0.7	1.8±0.2

from mantle to viscera in both Antarctic and Mediterranean molluscs. Lipids from mantle of Antarctic animals contained the highest levels of polyunsaturated fatty acids (PUFA) counting for ca. 50%. Also, the fatty acid composition varied considerably in SL and PL of the same individual (Table III).

In general, the SL of the mantle of Antarctic molluscs consisted largely of saturated (SFA) and monounsaturated (MUFA) components, with high SFA/PUFA and MUFA/PUFA ratios (Table V). Particularly, the SFA/PUFA ratio in SL of Mediterranean species was significantly lower than for Antarctic species. The PL of the mantle of Antarctic molluscs were featured by a high content of PUFA, with similar ratios for the SFA/PUFA and MUFA/PUFA ratios (Table V). Accordingly, the analysis of the SL composition of the mantle of Antarctic molluscs revealed that palmitic acid (16:0) and stearic acid (18:0) were the main acyl residues, whereas arachidonic acid (20:4 ω6) and eicosapentaenoic acid (20:5 ω3) were the dominant species in PL (Fig. 1). Compared to Antarctic gastropods, the mantle PL of Mediterranean molluscs contained higher levels of MUFA (Table V), with a particularly large content of octadecaenoic acid (15.98±12.4; 18:1 ω7/ω9) (Fig. 1). The MUFA/PUFA ratio was significantly higher than the Antarctic value (Table V).

Mantle sections of *T. challengeriana* contained levels of palmitoleic acid (16:1 ω7) considerably higher than those of palmitic acid and stearic acid, but the other two Antarctic species revealed an opposite composition (Table III). A similar trend was found in the viscera (Table IV). The overall percentage of PUFA, mainly arachidonic and eicosapentaenoic acids, was very similar in mantle PL from *M. mollis, B. clavigera* and *T. challengeriana* (49.2%, 44.3% and 58.0%, respectively) although the specific composition varied according to the species (Table III).

The extracts of the viscera contained similar amounts of FFA in Antarctic and Mediterranean animals (58.1±17.2 µg and 61.1±14.1 µg *per* mg of lipid extract, respectively). However, the content of FFA in the mantle was consistently higher in the Antarctic organisms (226.9±24.4 µg and 62.5±18.1 µg *per* mg of lipid extract, respectively in Antarctic and Mediterranean molluscs). The GC-

Table V. Fatty acid ratios in mantle SL (storage lipids: triglycerids and wax esters) and PL (phospholipids) in Antarctic and Mediterranean gastropods. SFA: Saturated fatty acids; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids. *: significantly different than the Antarctic species value ($p \leq 0.01$; t-test).

*Tabla V. Relación de ácidos grasos en SL del manto (lípidos de almacenamiento: triglicéridos y ésteres de ceras) y PL (fosfolípidos) en gasterópodos Antárticos y Mediterráneos. SFA: Acidos grasos saturados; PUFA: ácidos grasos poliinsaturados; MUFA: ácidos grasos monoinsaturados. *: significativamente diferente del valor obtenido en especies Antárticas ($p \leq 0.01$; t-test).*

	SL		PL	
	Antarctic	Mediterranean	Antarctic	Mediterranean
SFA/PUFA	2.45	0.39*	0.40	0.52
MUFA/PUFA	2.68	1.24	0.47	1.78*

MS analysis of mantle FFA showed a trend in fatty acid distribution similar to that found in mantle PL, with SFA and MUFA predominant in Mediterranean animals and PUFA more abundant in Antarctic organisms (Fig. 2). However, the fatty acid composition of mantle FFA in Antarctic molluscs proved to be rather different from that found in mantle PL and SL of the same species (Table III). Also the FFA profile in the mantle of Mediterranean molluscs showed very few similarities to the fatty acid distribution in FFA, PL and SL from the viscera of the same species (Tables III and IV). Analysis of FFA composition revealed that arachidonic acid (20:4 ω6), eicosapentaenoic acid (20:5 ω3) and docosahexaenoic acid (22:6 ω3) predominated significantly in Antarctic molluscs ($p<0.05$; t-test) whereas Mediterranean animals were mainly featured by a higher percentage of octadecadienoic acid (13.75±0.98; 18:2 ω6) which was almost absent in Antarctic animals (0.76±0.66) (Fig. 2).

DISCUSSION

In this study we analyzed the lipid composition of the opisthobranchs *B. clavigera*, *T. challengeriana*, *H. picta* and *D. limbata*, and the prosobranch *M. mollis*. Although the data are of limited value due to the few number of individuals analyzed from Antarctica, we believe that the results provide useful information on their fatty acid composition, distribution and comparison with Mediterranean species.

The total lipid content was very similar in the viscera of Antarctic and Mediterranean molluscs, but it proved to be higher in mantle sections of Mediterranean animals respect to the Antarctic ones. May be this difference could be related to the abundance in the Mediterranean species of non-polar components, such as terpenoids (ÁVILA, CIMINO, FONTANA, GAVAGNIN, ORTEA AND TRIVELLONE, 1991; ÁVILA, CIMINO, CRISPINO AND SPINELLA, 1991) that were less abundant in the extracts of the Antarctic species studied here.

T. challengeriana, contrary to the other Antarctic species, contained levels of palmitoleic acid (16:1 ω7) in mantle considerably higher than those of palmitic acid and stearic acid. As a similar trend was found in the viscera, we believe this may reflect dietary preferences. Lipid biomarkers have been recently used to clarify Antarctic trophodynamics in krill (see PHLEGER, NELSON, MOONEY AND NICHOLS, 2002). Perhaps further studies in Antarctic opisthobranchs will also help to understand their poorly known trophic relationships with other benthic organisms.

In theory, lipids from the viscera should be more dependent on factors

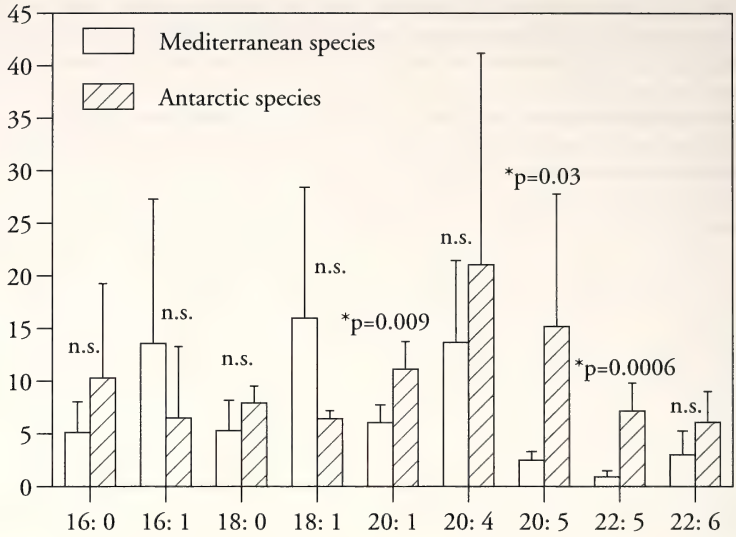

Figure 1. Relative percentage (mean ± SD) of phospholipid fatty acids (PL) in the mantle of the studied gastropod molluscs from the Antarctic and the Mediterranean. Statistical differences were determined by t-tests. *: p<0.05. n.s.: not significant. Acids: 16:0 palmitic acid, 16:1 palmitoleic acid, 18:0 stearic acid, 18:1 octadecaenoic acid, 20:1 eicosanoic acid, 20:4 arachidonic acid, 20:5 eicosapentaenoic acid, 22:5 docosapentaenoic acid, 22:6 docosahexaenoic acid.

*Figura 1. Porcentaje relativo (media ± SD) de ácidos grasos asociados a fosfolípidos (PL) en el manto de los moluscos gasterópodos estudiados de la Antártida y del Mediterráneo. Las diferencias estadísticas se determinaron mediante t-tests. *: p<0,05. n.s.: no significativo. Nombres de los ácidos: 16:0 ácido palmítico, 16:1 ácido palmitoleico, 18:0 ácido esteárico, 18:1 ácido octadecaenoico, 20:1 ácido eicosanoico, 20:4 ácido araquidónico, 20:5 ácido eicosapentaenoico, 22:5 ácido docosapentaenoico, 22:6 ácido docosahexaenoico.*

such as diet and reproductive cycles, whereas fatty acid composition of mantle should be far more responding to environmental conditions, such as temperature or depth. The fatty acid composition in SL and PL of mantle extracts were approximately similar in all animals studied, although the mantle PL of Antarctic species showed a higher content of unsaturated fatty acids (Fig. 1). In fact, the overall levels of PUFA in PL were similar in the three species of Antarctic molluscs, but were consistently higher than those of molluscs from the Mediterranean. MUFA/PUFA ratios in mantle PL were significantly divergent in Antarctic and Mediterranean molluscs, and suggested a different composition of membrane phospholipids from the two groups. In fact, fatty acids in PL have positional specificity, being the *sn*-1 and *sn*-2 position of glycerol usually occupied by saturated (or trans-unsaturated) and polyunsaturated groups, respectively. At a molecular basis, the fatty acid composition of the mantle of Antarctic specimens supported the presence of a large fraction of PL with polyunsaturated/polyunsaturated or monounsaturated/polyunsaturated chains, whereas the analysis of the Mediterranean specimens indicated an opposite trend in PL with saturated or monounsaturated species at both the 1-position and the 2-position. In a study on fish (Dey *et al.*, 1993), it was suggested that some phospholipids, such as those containing oleic/docosahexaenoic and oleic/eicosapentaenoic acids, play an important role in the membrane

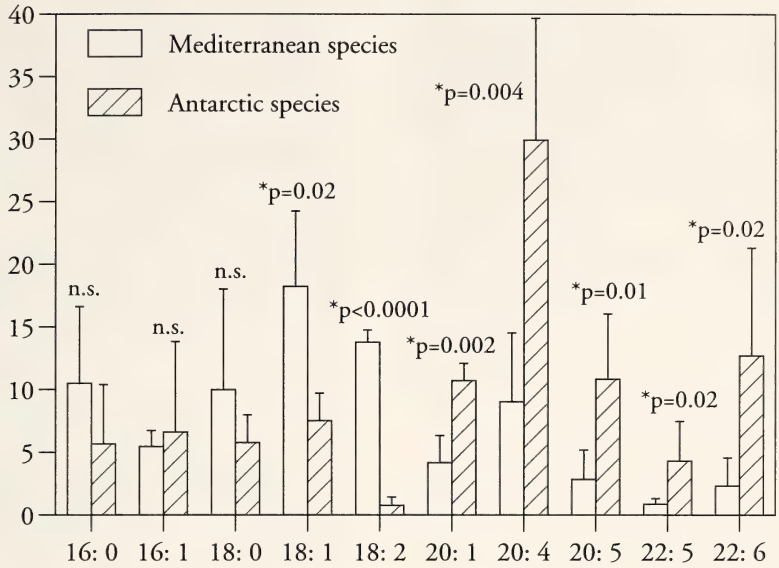

Figure 2. Relative percentage (mean ± SD) of free fatty acids (FFA) in the mantle of the studied gastropod molluscs from the Antarctic and the Mediterranean. Statistical differences were determined by t-tests. *: p<0.05. n.s.: not significant. Acids are as in Figure 1, and 18:2 is octadecadienoic acid.
*Figura 2. Porcentaje relativo (media ± SD) de ácidos grasos libres (FFA) en el manto de los moluscos gasterópodos estudiados de la Antártida y del Mediterráneo. Las diferencias estadísticas se determinaron mediante t-tests. *: p<0,05. n.s.: no significativo. Nombres de los ácidos como en la Figura 1, y 18:2 es el ácido octadecadienoico.*

homeostasis. In particular, an increase of the unsaturated fatty acid percentage in the *sn*-1 position of phospholipids, such as that due to the replacement of palmitic acid by oleic acid, may affect the membrane structure in order to maintain its functional integrity at cold temperatures (DEY *ET AL.*, 1993). Our data, therefore, are in agreement with this, having found high levels of polyunsaturated or monounsaturated fatty acids in position *sn*-1 of mantle phospholipids in Antarctic animals.

Our results also showed a very high content of FFA in the Antarctic samples. Free fatty acids may be produced due to degradation of SL and PL during handling and storage. Consequences of slow frozen storage autolysis are well know in fish research (HARDY, MCGILL AND GUNSTONE, 1979) and these may have affected our FFA results, since our procedure consisted in a very fast storage of samples at –30°C until extraction, while only a storage a –80°C completely blocks enzymatic activity. However, there are some evidences suggesting a physiological meaning for these FFA levels. First, degradation did not occur at a similar rate in mantle and viscera of the animals, since we detected higher quantities of FFA in the mantle. Moreover, no apparent correlation was observed between the FFA composition of the mantle and that of the other lipid pools from both mantle and viscera. We believe that the production and occurrence of high levels of FFA may be a distinct characteristic of these Antarctic species, reflecting the chemical-physical properties of the cold-adapted metabolism of these organisms (e.g. lipases). Further research should investigate this possibility with larger numbers of specimens.

Besides the reported high amounts, the specific composition of FFA in the mantle of Antarctic and Mediterranean molluscs was also remarkably different (Fig. 2). While Antarctic species were characterized by higher levels of PUFA, Mediterranean animals showed a dominance of saturated and monounsaturated species. It seems probable that the different distribution of FFA may indicate an environmental adaptation. Whether and how the accumulation of FFA is related in any way to the membrane homeostasis remains to be thoroughly investigated. It is interesting to note, however, that the high concentration of FFA can be one of the ways to transcend the positional specificity of fatty acids in phospholipids (MEAD, ALFIN-SLATER, HOWTON AND POPJÀK, 1986). In fact, the formation of phospholipids involves the transfer of an acyl group from CoA to either *sn*-1 or *sn*-2 positions of the corresponding lysophosphoglycerides. Such transacylation is catalysed by acyltransferases, which are enzymes sensitive to the chemical features of the fatty acid chains. As discussed above, the final result of this preference is the positioning of saturated fatty acids at the 1-position and of *cis*-unsaturated fatty acids at the 2-position. It has been demonstrated that the position-dependent specificity of acyltransferases can be overridden by the fatty acid concentration (MEAD *ET AL.*, 1986). In the Antarctic molluscs, the presence of high levels of PUFA, therefore, may be needed for the synthesis of phospholipids with polyunsaturated chains at both the 1-position and the 2-position of glycerol. Further studies are, however, necessary to clarify this point.

Our results show that gastropod molluscs, and opisthobranchs in particular, possess biochemical mechanisms of adapting the fatty acid composition of phospholipids to temperature. Similarly, Viarengo and coworkers (VIARENGO, ACCOMANDO, ROMA, BENATTI, DAMONTE AND ORUNESU, 1994) demonstrated that fatty acid differences between Antarctic and Mediterranean scallops were related to cell membrane adaptation to low temperatures. Although the number of species investigated in our study is very limited, we suggest that these data are representative of the lipid adaptation in gastropod molluscs from cold and temperate environments.

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Why are beached *Acanthochitona fascicularis* (Linnaeus, 1767) (Mollusca: Polyplacophora) from Italy missing their dorsal girdle elements?

¿Por qué los ejemplares de *Acanthochitona fascicularis* (Linnaeus, 1767) (Mollusca: Polyplacophora) encontrados en una playa de Italia pierden los elementos del perinoto?

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ABSTRACT

An unusual form with reduced dorsal girdle of the well known and extremely variable European chiton *Acanthochitona fascicularis* (Linnaeus, 1767) is hereby illustrated, discussed, and compared with the regular form. Hundreds of beached specimens and several live found specimens with similar dorsal girdle were studied and compared. Different theories and hypothesis are proposed as an explanation for this unusual form and as a conclusion we finish with some open questions.

RESUMEN

En el presente trabajo se estudia una forma inusual con reducción de elementos del perinoto del poliplacóforo *Acanthochitona fascicularis* (Linnaeus, 1767). Se compara, se ilustra y se discuten los datos obtenidos con la forma regular de esta especie. Los autores aportan su hipótesis sobre la presencia de esta inusual forma y concluyen que hay algunas cuestiones abiertas.

KEY WORDS: Polyplacophora, *Acanthochitona fascicularis*, Mediterranean Sea, girdle.
PALABRAS CLAVE: Polyplacophora, *Acanthochitona fascicularis*, Mediterráneo, perinoto.

INTRODUCTION

Many years ago, a great number of specimens of *Acanthochitona fascicularis* (Linnaeus, 1767) were beached after a storm at "Calambrone", a locality near Livorno (Tuscany, Italy). A friend of the first author picked up many of these and prepared part of them in an alcohol/glycerine solution, the rest were preserved in alcohol. All collected specimens had an unusual feature in common: a "smooth" dorsal girdle with reduced sutural tufts. Later on, similar

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living specimens were found at two localities in Italy, which made a comparative study possible.

Abbreviations:

BDA Private Collection of Bruno Dell'Angelo, Italy.

BA Private Collection Bruno Anseeuw, Belgium.

YT Private Collection Yves Terryn, Belgium.

MATERIAL AND METHODS

Material examined: *Acanthochitona fascicularis* (Linnaeus, 1767)

• Calambrone, Livorno prov., Italy, 56 specimens beached in Feb. 1991 (BDA, Fig.7), including 21 preserved in alcohol (Fig.6). The dimensions vary from 28 x 15 mm to 40.5 x 18.5 mm. Only one spm is of smaller dimensions, 13 x 6.8 mm.

• Calambrone, Livorno prov., Italy, 5 specimens beached on Feb. 1991 (BDA), about 28.5 x 14 mm, with plates covered with Bryozoa and other organisms (Fig.5).

• Punta Faro, Messina Strait, Italy, under rocks at 2-3 m: 2 specimens, about 25 x 14 mm (estimated) strongly curled and 18 x 12 mm curled (BDA).

• Off Capraia Isl., Italy, dredged at about -200 m, inside a large semi porous rock (about 70 x 70 cm): 5 specimens live taken (S. Savona collection, Italy).

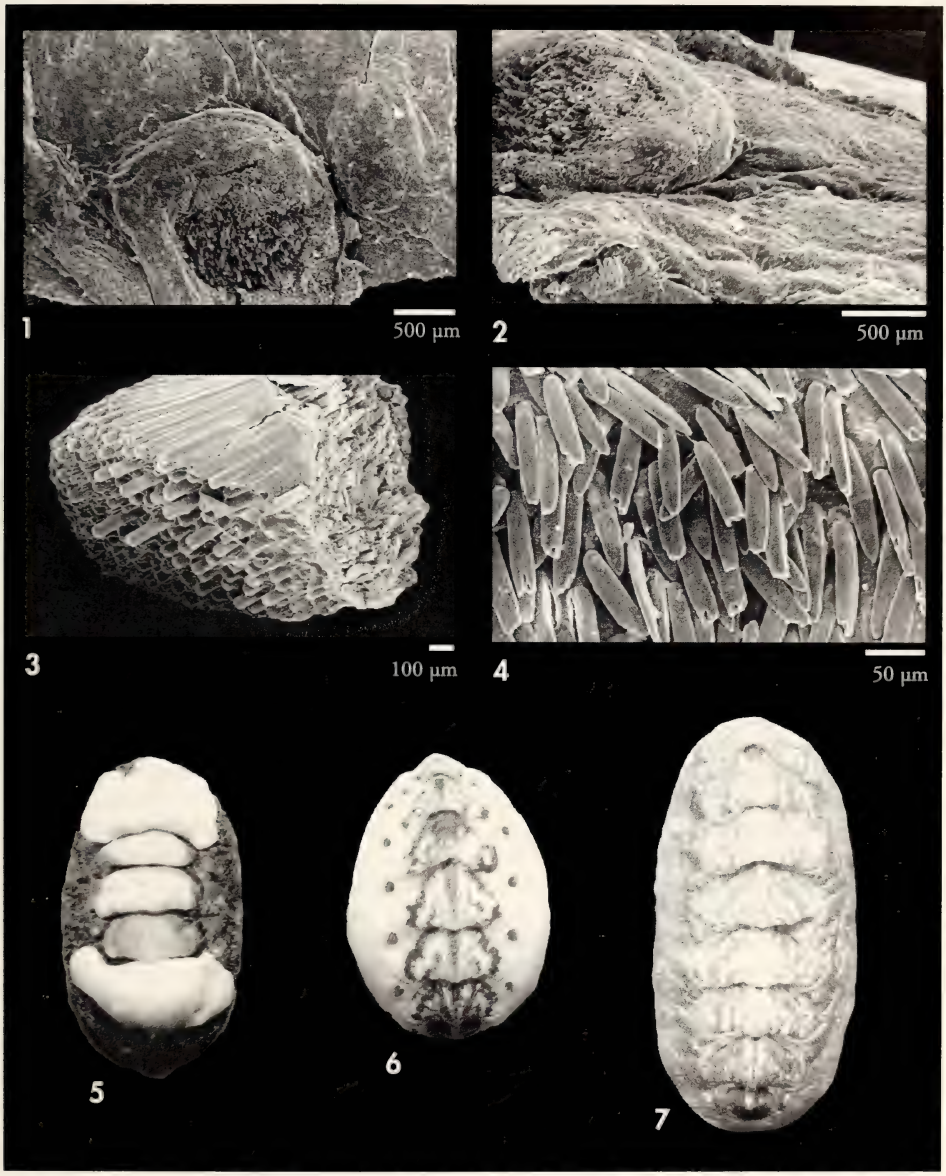
• Hundreds of specimens from diverse European (Portugal, Spain, France, Italy, Croatia, Greece) and African (Morocco, Algeria, Tunisia, Israel) localities (BDA, BA and YT collections).

SEM-images were made using a SEM *JEOL* mod. JSM-5200. Specimens were disarticulated by boiling in a KOH-solution until all soft parts were dissolved, followed by thorough rinsing.

RESULTS

The mollusc-beaching phenomenon at Calambrone is well known (Campani, 1983), and the number of molluscs species beached over a period of several years is rather high (257 species). The beaching is caused by interaction between different sea currents and by the characteristic sea floor. Among these beached molluscs, three species of chitons have been reported: *Ischnochiton rissoi* (Payraudeau, 1826), *Chiton olivaceus* Spengler, 1797 and *Acanthochitona fascicularis* (Linnaeus, 1767) (reported in Campani's list as *Acanthochitona aenea*). The beaching of chitons is generally uncommon, and usually concerning few specimens. The beaching in February 1991 is therefore particularly significant especially as the features of the *A. fascicularis* beached are so different from typical shallow-water *A. fascicularis*. Some of these specimens have been illustrated in the book on chitons from the Mediterranean Sea (Dell'Angelo and Smriglio, 2001).

When we first saw these beached "smooth" specimens, we thought they were regular *A. fascicularis* with damaged dorsal girdle. In fact, it seemed as if the dorsal girdle elements had been removed by some cause as all spicules were gone and the sutural tufts, which normally bear long spicules, were very short , almost truncate. Beside this, all other features looked macroscopically identical to normal *A. fascicularis*, i.e. general shape and tegmentum sculpture. As these specimens were dead, some external influence could have caused this phenomenon. For example the specimens could have died during the storm resulting in curled up specimens which could have been dorsally damaged by scraping and rolling upon rocks during the beaching process. This could explain why only the dorsal girdle was removed and not the ventral part which seemed to be intact. However, the tegmentum showed no obvious signs of damage. It was as if something had meticulously removed the dorsal girdle, without touching the rest of the animal. We deliberately removed the dorsal girdle elements (by scraping with a knife) from a similar "regular", alcohol/glycerine preserved specimen and the result was a specimen



Figures 1-4. *Acanthochitona fascicularis* beached, with "smooth" dorsal girdle. 1: girdle, tuft; 2: girdle, tuft, lateral view; 3: isolated tuft (right: top, left: inside the girdle); 4: girdle, ventral spicules. Figures 5-7. *Acanthochitona fascicularis* beached. 5: one of the five specimens with the plates covered with bryozoans and other organisms (28.2 x 14.3 mm); 6: one of the specimens preserved in alcohol (28.5 x 17.6 mm); 7: one of the specimens prepared in an alcohol/glycerine solution (38.7 x 17.5 mm).

Figuras 1-4. Acanthochitona fascicularis, *con el perinoto dorsal "liso". 1: perinoto, protuberancia; 2: perinoto, protuberancia, vista lateral; 3: protuberancia aislada (derecha: parte superior, izquierda: dentro del perinoto); 4: perinoto, espículas ventrales. Figuras 5-7.* Acanthochitona fascicularis. *5: uno de los cinco especímenes con las paclas cubiertas por briozoos y otros organismos (28,2 x 14,3 mm); 6: especimen conservado en alcohol (28,5 x 17,6 mm); 7: especimen preparado en solución de alcohol/glicerina (38,7 x 17,5 mm).*

that looked almost identical to the beached ones. The external cause remains therefore a possible explanation of the phenomenon.

The disarticulation of one of the specimens was necessary for a complete analysis and comparison with a typical specimen of *A. fascicularis*. When disarticulated no dorsal girdle appeared. Normally, when submitting a chiton to this process, the soft parts of the animal will dissolve and it will result in the 8 isolated valves, the radula and a thin layer of dorsal and ventral girdle bearing the girdle elements. In the beached specimen, only the ventral layer was present. The sutural tufts, which are normally attached to the dorsal layer, were loose. We concluded that the specimen had no dorsal girdle, but what caused this lack of girdle?

Other material beached at Calambrone during February 1991 provided new insight into this problem. Among the molluscs beached were five specimens of *A. fascicularis* with valves severely covered with corals and/or eroded, but with the dorsal girdle in normal condition (Fig. 5). The only difference from regular *A. fascicularis* is the reduced tufts, but this is known to occur within members of this species. Also, several of the alcohol preserved specimens still have some of their dorsal girdle elements present (i.e. areas with fine and short spicules) and reduced tufts, which supported the notion that the lack of dorsal girdle in the previous material was, in fact, caused by external influences.

DISCUSSION

We have considered several hypotheses that might explain the phenomenon of beached *Acanthochitona "fascicularis"* with reduced or detached dorsal girdles. First, it is possible that the specimens were subjected to an acidic environment, either before or after their death. The problem is that this acidic environment would also "etch" the tegmentum and there is no evidence of this visible on the specimens. We consider this hypothesis unlikely. Another possible cause is that the specimens lived below the carbon compensation depth, where seawater dissolves calcareous material quite aggressively, but this is only known to appear at great depths (>4000 m) so this can be rejected, as the specimens were found on the shore and it is highly improbable that they were transported from great depths by the storm.

Could this phenomenon have been caused by polluted water? Is it perhaps caused by something similar as the "red tides" which are known to kill chitons in great numbers in South Africa for example? (*fide* A. Seccombe, *pers.com.* 1997; B. Anseeuw and Y. Terryn, *pers.obs.* 2002: beaching of *Dinoplax gigas* Gmelin, 1791). Has an animal removed the dorsal girdle?

One possible hypothesis is that the specimens could be a distinctive ecological form (ecotype) of *fascicularis*, i.e. with reduced dorsal girdle, or even an unknown species. We are unaware of any previous mention of this distinction in literature regarding chitons. In fact, it is highly improbable that a species could live without their protecting dorsal girdle, so this hypothesis was also considered as highly improbable. It is however known that the dorsal girdle elements can be quite variable, even within specimens from the same locality. For example specimens from a locality which are found on exposed habitats can have reduced dorsal girdle elements, but will also generally show eroded tegmentum, a feature that was not observed in the beached specimens.

The most logical remaining hypothesis for us was that some unknown posthumous process led to the removal of the dorsal girdle. However, we were then at a loss to explain our later observation of living specimens with similar "naked" appearance. This fact made everything much more complicated as these specimens were alive but without a dorsal girdle. Thus, we still do not have an explanation about why all these specimens have a reduced dorsal girdle. It is also possible that these specimens could represent a species or race that is distinct from typical *A. fascicularis* but the only

difference between both that we have observed so far is the absence of the dorsal girdle elements. On the other hand, it is highly improbable that a lineage of chitons would lose their dorsal girdle, as it would seem to make it quite unprotected and vulnerable. Perhaps a DNA analysis could test the "different species" hypothesis but this can only be done easily on fresh alcohol-preserved specimens. Perhaps the future will bring some fresh alcohol-preserved specimens but until then we can only speculate. We would be happy to hear of anyone who has observed similar specimens or of similar observations for other chiton species, as more information could eventually lead to a plausible explanation.

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The genus *Calyptraea* (Gastropoda, Caenogastropoda, Calyptraeidae) in the East Atlantic

El género *Calyptraea* (Gastropoda, Caenogastropoda, Calyptraeidae) en el Atlántico oriental

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ABSTRACT

The genus *Calyptraea* is revised for the East Atlantic. Up to now, only *C. chinensis* was recognized but this species is only present in the Mediterranean Sea, and in the European and North African Atlantic. Two new African species confused up to now with *C. chinensis* are described, one with a range from Western Sahara to Guinea Conakry and Benin, and the other from Mauritania to Angola. Another South African species, also considered *C. chinensis*, is in fact a different taxon.

RESUMEN

Se revisa el género *Calyptraea* en el Atlántico oriental. *Calyptraea chinensis*, la única especie reconocida actualmente, se distribuye sólo en el Mediterráneo, y en el Atlántico europeo y norteafricano. Dos nuevas especies, hasta ahora consideradas como *C. chinensis*, se encuentran en África; una de ellas se extiende desde el Sáhara Occidental hasta Guinea Conakry y Benin, y otra desde Mauritania hasta Angola. Otra especie de Sudáfrica, también considerada hasta el momento como *C. chinensis*, es una especie diferente.

KEY WORDS: *Calyptraea chinensis*, Europe, West Africa, new species.
PALABRAS CLAVE: *Calyptraea chinensis*, Europa, África occidental, nuevas especies.

INTRODUCTION

The genus *Calyptraea* Lamarck, 1799 is represented in the East Atlantic by only one species, in the opinion of most recent authors (NICKLÈS, 1950; BERNARD, 1984; FRETTER AND GRAHAM, 1981; SABELLI, GIANNUZZI-SAVELLI AND BEDULLI, 1991; POPPE AND GOTO, 1991; ROLÁN AND RYALL, 1999 and CHIARELLI, 2002). Against this recent situation, more species of this genus are known from the fossil records (CASTAÑO, CIVIS AND GONZÁLEZ DELGADO, 1988; VERA-PELÁEZ, LOZANO-FRANCISCO, MUÑIZ-SOLÍS, GILI, MARTINELL, DOMÈNECH, PALMQVIST AND GUERRA-MERCHÁN, 1995). The systematics and the phylogeny of the genus *Calyptraea* were studied by FISCHER (1950).

Being a common species with a wide distribution, it was to be expected that some of the names employed in the past to designate *C. chinensis* are actually synonyms (most from the Mediterranean) (see SABELLI *ET AL.*, 1991 and below).

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A general description of this species is given in FRETTER AND GRAHAM (1962, 1981) together with bibliographic information on different aspects. Other references to this species will be mentioned below under distribution.

After studying numerous samples of material from many European and West African localities, the comparison of shells, anatomy and other characters indicated the existence of more than one species. This is the subject of the present work.

MATERIAL AND METHODS

Some of the material examined as well as numerous specimens and shells were obtained by the author from expeditions to several West African localities: Morocco, Mauritania, Canary Islands, Senegal, Ghana, São Tomé Is., Principe Is., Annobón Is. and Angola. Many samples from the MNHN collection and also from some private collections mentioned below were also studied. *In vivo* observations were made by the author.

Abbreviations

AMNH American Museum of Natural History, New York
BMNH The Natural History Museum, London
IPM Instituto Português de Malacologia, Lisbon
LS Linnean Society, London
MNCN Museo Nacional de Ciencias Naturales, Madrid
MNHN Muséum Nationale d'Histoire Naturelle, Paris
ZSM Zoologisch Staatssammlung, München
CJH collection of José María Hernández, Gran Canaria
CER collection of Emilio Rolán, Vigo
CFS collection of Frank Swinnen, Lommel, Belgium
sp specimen with soft parts
s shell
f fragment
j juvenile. As the smaller specimens are adult males (BACCI, 1951), only in very small shells was the category of juvenile employed.

RESULTS

Family CALYPTRAEIDAE Lamarck, 1809
Genus *Calyptraea* Lamarck, 1799

Calyptraea chinensis (Linnaeus, 1758) (Figs. 1-27, 65-67, 83-85, 91, 94, 97, 100, 103-107, 113-116)

Patella chinensis Linnaeus, 1758. *Syst. Nat.*, ed. X, p. 781, no. 656; ed. XII, p. 1257, no. 749. [Type locality: M. Mediterraneo].
Patella sinensis Gmelin, 1791. *Syst. Nat.*, ed. XIII, p. 3692.
Patella albida Donovan, 1802. *Nat. hist. Brit. Shells*, IV, pl. 129.
Patella squamulata Renier, 1804. *Prodr. Observ.* [not available: published in a paper rejected by ICZN opinion 316/1954].
Patella muricata Brocchi, 1814. *Conch. foss. subap.*, p. 16.
Calyptraea laevigata Lamarck, 1822. *Hist. nat. anim. sans vert.* VI, II, p. 21.
Calyptraea succinea Risso, 1826. *Hist. Nat. Eur. Mer.*, p. 255.
Calyptraea vulgaris Philippi, 1836. *Enum. moll. Siciliae*, I, p. 119.
Calyptraea polii Scacchi, 1836. *Catal. Conch. Regn. Neapolitani*, p. 17.
Calyptraea spirata Nardo, 1847. *Sinon. modern...Golfo Veneto*. In SABELLI ET AL. (1990), p. 160.
Calyptraea canarica Buonanni *fide* JEFFREYS (1865). *Brit. Conch.*, 3, p. 275.
Calyptraea mamma Krynicki *fide* Middendorff in JEFFREYS (1865). *Brit. Conch.* 3, p. 276.
Calyptraea polii var. *fusca* Issel, 1878. *Croc. del Viol.*-Testacei, p. 31.
Calyptraea sinensis var. *coralligena* Pallary, 1900. *Coquil. marins d'Oran*, p. 329.
Calyptraea sinensis var. *elliptica* Pallary, 1900. *Coquil. marins d'Oran*, p. 329.

Type material: 2 syntypes, in LS (Figs. 113-116); the larger one is here designated lectotype (Figs. 113, 114)

Other material examined: Great Britain: 36 s, Shells Bay, Studtland, Dorset (MNHN). Atlantic France: 15 s, Saint Michel, Normandy, in shells on sand, intertidal (CER); 15 sp, 16 s, 10 j, Les Cochons Noires, Roscoff, Finistère, 20 m (MNHN); 4 sp, Roscoff, on shells of sand bottom, intertidal (CER); 3 c, NE canyon de la Cassidaigne 48° 06.7′ N 05° 55′ E, 150-250 m (MNHN); 35 sp, Saint Quay Portrieux, Baie de Saint Brieuc, 10-30 m, on shells (CER); 4 s, Plougastel, Tinduff, port (MNHN); 33 s, Anse de Bertheaume, 20-30 m (MNHN). Atlantic Spain: 40 sp, Ribadeo, intertidal, on shells (CER); 12 sp, 23 s, Vigo, Samil, 12 m, on shells and rocks (CER); 155 sp, 30 s, O Grove, on shells, sandy bottom, intertidal (CER); 53 sp, 30 s, Vigo, Cies Islands, 9 m, on shells and rocks (CER); 20 sp, Punta do Arroás, Vigo, intertidal (CER); 3 s, Vigo, San Simón, 5 m, on shells, sandy bottom (CER); 55 sp, Vigo, Toralla, 20 m, on shells, sandy bottom (CER); 3 j, Conil de la Frontera (MNHN); 6 s, Cádiz Bay, 18 m, from sandy sediment (CER); 1 c, El Puerto de Santa María, Cádiz, beach sediment (MNHN); 6 s, Sotogrande, Torre Guadiaro, Cádiz, circalittoral (MNHN); 15 c, Torre de la Peña, Tarifa, in beach sediment (MNHN). Portugal: 30 sp, 9 s, Tavira, Terra Estreita 37° 06′ N 07° 38.4′ W, 3 m (MNHN); 2 sp, Ria Formosa, Ilha do Farol, Casi dos Hangares, 3 m (IPM); 6 sp, 7 s, Algarve, between Sagres and Faro, 40-50 m (MNHN); 106 sp, 70 c, Chenal do Olhão, 37° 00′ N 07° 51′ W, 3-7 m (MNHN); 6 sp, 12 s, Baia de Baleeira, 37° 00.7′ N 8° 55′ W, 12-17 m (MNHN); 20 sp, 8 s, Baia do Peixe, 37° 00′ N 08° 58′ W, 23 m (MNHN); 1 sp, Algarve 36° 56′ 871″N 08° 17′ 45″W, 69-105 m (IPM). Mediterranean Spain: 114 sp, 165 s, La Atunara, La Línea de la Concepción, 27 m, on shells, in sandy bottom (CER); 2 s, La Atunara Beach, La Línea, 36° 10.2′ N 05° 19.3′ W, 30 m (MNHN); 1 s, La Atunara Beach, La Línea, 36° 05.19′ N 05° 19′ W, 40-45 m (MNHN); 15 s, Málaga, 20-40 m (MNHN); 5 sp, 23 s, 30 j, Rincón de la Victoria, Málaga (MNHN); 4 s, Marbella port (MNHN); 3 s, Marbella (CJH); 1 c, Tarajal, Ceuta (MNHN). Mediterranean France: 5 c, Lion Gulf, N/O "Europe" IFREMER/DEPRO 96, 42° 20′ N 03° 27.1′ W, 650-725 m (MNHN); 2 sp, Lacaze-Duthiers, Banyuls-ECOMARGE, 42° 30′ N 03° 25.20E, 130-190 m (MNHN); 2 sp, Cap Béar, Banyuls-ECOMARGE, 42° 29.40′ N 03° 10.40E, 66-67 m (MNHN); 1 sp, Cap Béar, Banyuls-ECOMARGE, 42° 30′ N 03° 10.50E, 62 m (MNHN); 7 sp, 1 sp, Cap Béar, Banyuls-ECOMARGE, 42° 31′ N 03° 10.10E, 70 m (MNHN); 39 s, Marseille N/O "Georges Petit", Grand Congloué, 83 m (MNHN); 7 s, Le Brusc/Cap Sicié, Provenza, 40-100 m (MNHN). Morocco: 13 c, Playa Grande, Tanger (MNHN); 1s, Essaouira, 31° 31′ N 09° 47′ W, beach sediment 2 sp, Cap Béar, Banyuls-ECOMARGE, 42° 29.40′ N 03° 10.40E, 66-67 m (MNHN); 1 c, 16 j, N/O Vanneau, 30° 40′ N 04° 55′ W, 20 m (MNHN); 3 sp, 4 c, 7 j, N/O Vanneau, 30° 30′ N 09° 43′ W, 40 m (MNHN); about 300 s and j, N/O Vanneau, 34° 54′ N 09° 58′ W, 110 m (MNHN); 4 s, N′ Dig (Rincon), circalittoral (MNHN); 3 s, 10 km N Agadir (CFS). Algeria: 3 s, (MNHN); 1 sp, 1 s, 1 j, Oran (MNHN). Italy: 4 c, Tuscan archipelago (MNHN); 12 s, Naples (MNHN); 2 s, Palermo and Messina (MNHN). Tunis: 3 s, Canal d′ Ajim, Djerba, 10-32 m (MNHN); 56 c, Gulf of Gabes, 10-15 m (MNHN). Rumania: 4 sp (MNHN). Madeira: 19 s, Pontinha-Cais do Lazareto, 60 m (CFS); 14 s, Lido, Funchal (CFS); 26 c, Funchal Bay (CFS); 15 s, Funchal Bay, 25-30 m (CFS); 22 s, Funchal Bay, 50 m (CFS); 26 s, 1 f, Funchal Bay, 70-80 m (CFS); 12 s, Funchal Bay, 100 m (CFS); 3 s, Funchal Bay, 130 m (CFS); 3 s, Funchal Bay, 150 m (CFS); 4 s, Funchal Bay, 180 m (CFS). Canary Islands: 2 s, Gando Bay, Gran Canaria, 15 m (CFS); 2 s, Maspalomas, Gran Canaria (CFS). Western Sahara: 6 s, S. Peña Grande, 24° 45′ N 15° 25′ W (CJH); 1 s, Villacisneros, 30 m (CJH).

Description: The original description of Linnaeus is: "*Testa integra subconica laevi labio laterali*". For a more complete description, see FRETTER AND GRAHAM (1981). The shell (Figs. 1-10) has been illustrated in many papers, but the colour variability was only well represented in GIANNUZZI-SAVELLI *ET AL.* (1997) and MACEDA *ET AL.* (1999). The protoconch (Figs. 11-26) is described in FRETTER AND GRAHAM (1981) as "having 0.75 to 1 smooth whorls but this is often eroded.... The protoconch is not clearly separated from the teleoconch. Its diameter is 600-700 µm". This description is correct but the nucleus of the protoconch, according to the definition of VERDUIN (1976), has between 194 and 230 µm. From this nucleus, the protoconch increases in size quickly to reach 3-3.5 times the diameter of the nucleus, and 0.75 to 1 whorl at its end, although the limit with the teleoconch is not clearly defined. The total diameter of the protoconch in the shells studied was between 630 and 741 µm. Seen from the

side the protoconch is situated on the teleoconch without a deep suture between them. Its surface is apparently smooth but, at high magnification (Figs. 19, 24, 26), numerous, short axial striae disposed in spiral bands may be observed. This description is valid for 98% of the more than three hundred protoconchs studied: for some variations, see below, in Remarks. The teleoconch is rather smooth, of a patelliform shape, rounded, rather solid, opaque and not glossy. Ratio between width and height is usually between 2.5 and 3.1. Only exceptional populations may have differences in shape, the shells being higher or flatter (see below). Many shells are smooth, not shiny, with or without prominences, sometimes with oblique dorsal threads near the border. Within the same populations, a variable number of shells show scarcely separated prominent spines (Figs. 10, 100).

The colour of the shell is most frequently white but it may be yellowish, cream, pink, brown or violet. The colour of each population is variable, with white or brown sometimes predominating.

Dimensions: Fretter and Graham (1981) indicated up to 15 mm, which is similar to the maximum dimension mentioned in most papers; Locard (1892) mentions up to 20 mm. After examining many specimens, it is uncommon for the species to reach more that 20 mm, but there is a shell (CER) from Pobra do Caramiñal, north-west Spain, with a diameter of 24.7 mm; in the MNHN there is another from Málaga of 25 mm and, finally, the record is in CJH where there is a shell from Malaga of 30 mm in diameter.

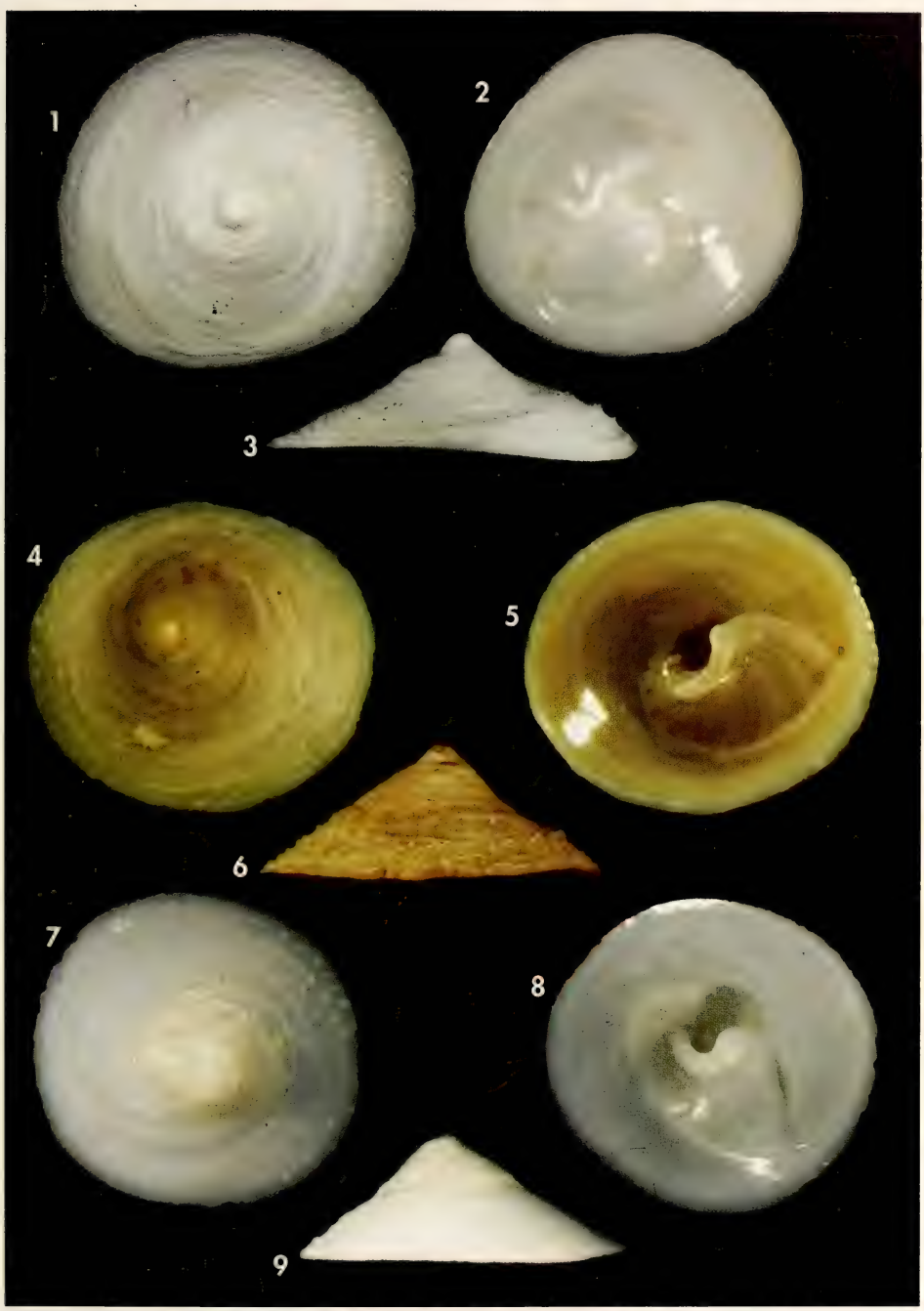
The animal was described in Fretter and Graham (1962, 1981). The Vigo and O Grove specimens which were studied live were whitish, with milk-white, large, irregular spots on the border of the mantle forming groups (Fig. 94). La Atunara (southern Spain) specimens studied preserved in alcohol had orange spots on the mantle border, with opaque translucent white and cream dots all around.

Due to the importance of the penis (Fig. 97) in comparison with other species, it should be described in more detail. It is placed behind the right tentacle and is elongate with a bilobed knob at its apex. These two lobes are different, the larger one being like a spoon, and the smaller one like a finger, with an arrangement similar to a hand with the four fingers fused and forming a concave prolongation, and the thumb in opposition.

The radula (shown in Bandel, 1984 and Finet, Wüest and Mareda, 1992) (Figs. 83-85, 91) is taenioglossate; rachidian tooth narrow and with a prominent central cusp with 4, sometimes 5, smaller cusps at each side. Lateral teeth with a prominent and wide cusp at the confluence of the internal and the external edges; this cusp may have serrate borders. Internal edge with few wide cusps (usually 4) while the external edge has between 7 and 12. Marginal teeth elongate, curved, sharp pointed and the internal tooth with two borders with few cusps (4-6), while the external one has few cusps (2-4) on its internal part.

The spawn of *C. chinensis* (Figs. 103-105) was studied by Lebour (1937), Wyatt (1957) and recently described by Troncoso, Urgorri and Cristobo (1988) in populations from north-west Spain. It is formed by numerous spherical-ovoid capsules, with a circular section (Figs. 103-104), which are fixed in groups to the substrate by a narrow peduncle (Fig. 105). The development of this species is direct; Troncoso *et al.* (1988) have shown the intracapsular veliger stage, and here we present two more advanced stages (Figs. 106, 107).

The numerical data in Troncoso *et al.* (1988) are the following: capsules 1.2-3.0 mm, number of eggs per capsule 2-18 and each egg 0.6-1.0 mm (the reason for this last size is that it refers to two larvae in a capsule). Specimens of 15-20 mm from Vigo and O Grove had 8-15 capsules per spawn, of 10-16 eggs each, 380-490 µm in diameter. From La Atunara, spawn from specimens of 12-17 mm had 8-18 capsules each, with 6-17 eggs each, of 340-430 µm in diameter.



Figures 1-9: *Calyptraea chinensis:* 1-3: Shell, 13.2 mm, Vigo (CER); 4-5: Shell, 13.5 mm, La Atunara, La Línea de la Concepción, Spain (CER); 6: Shell, 8.8 mm, Vigo, Spain; 7-9: Shell, 15.8 mm, St. Brieuc Bay, Bretagne, France (CER).

Figuras 1-9: Calyptraea chinensis*: 1-3: Concha, 13,2 mm, Vigo (CER); 4-5: Concha, 13,5 mm, La Atunara, La Línea de la Concepción, España (CER); 6: Concha, 8,8 mm, Vigo, España; 7-9: Concha, 15,8 mm, St. Brieuc Bay, Bretaña, Francia (CER).*

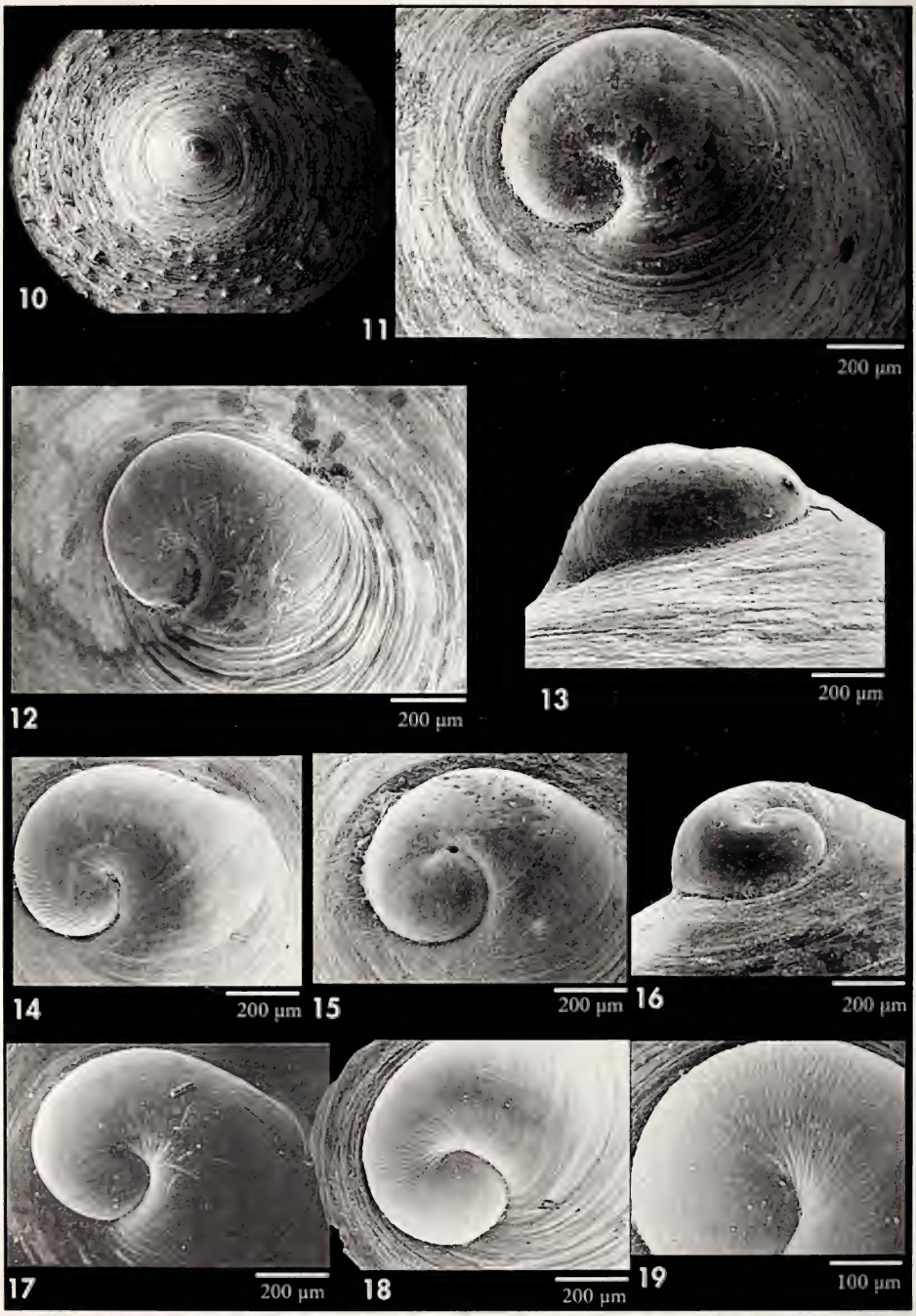
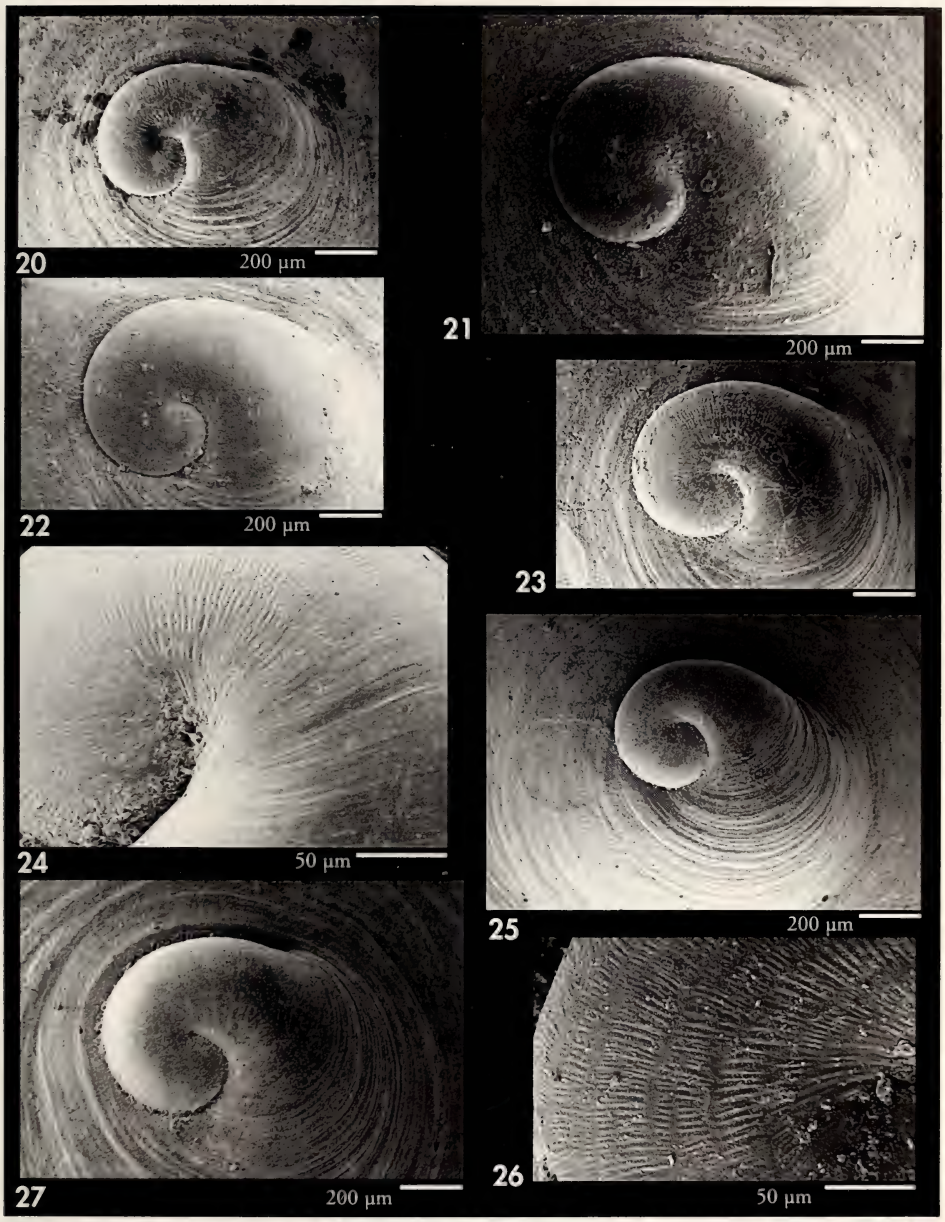
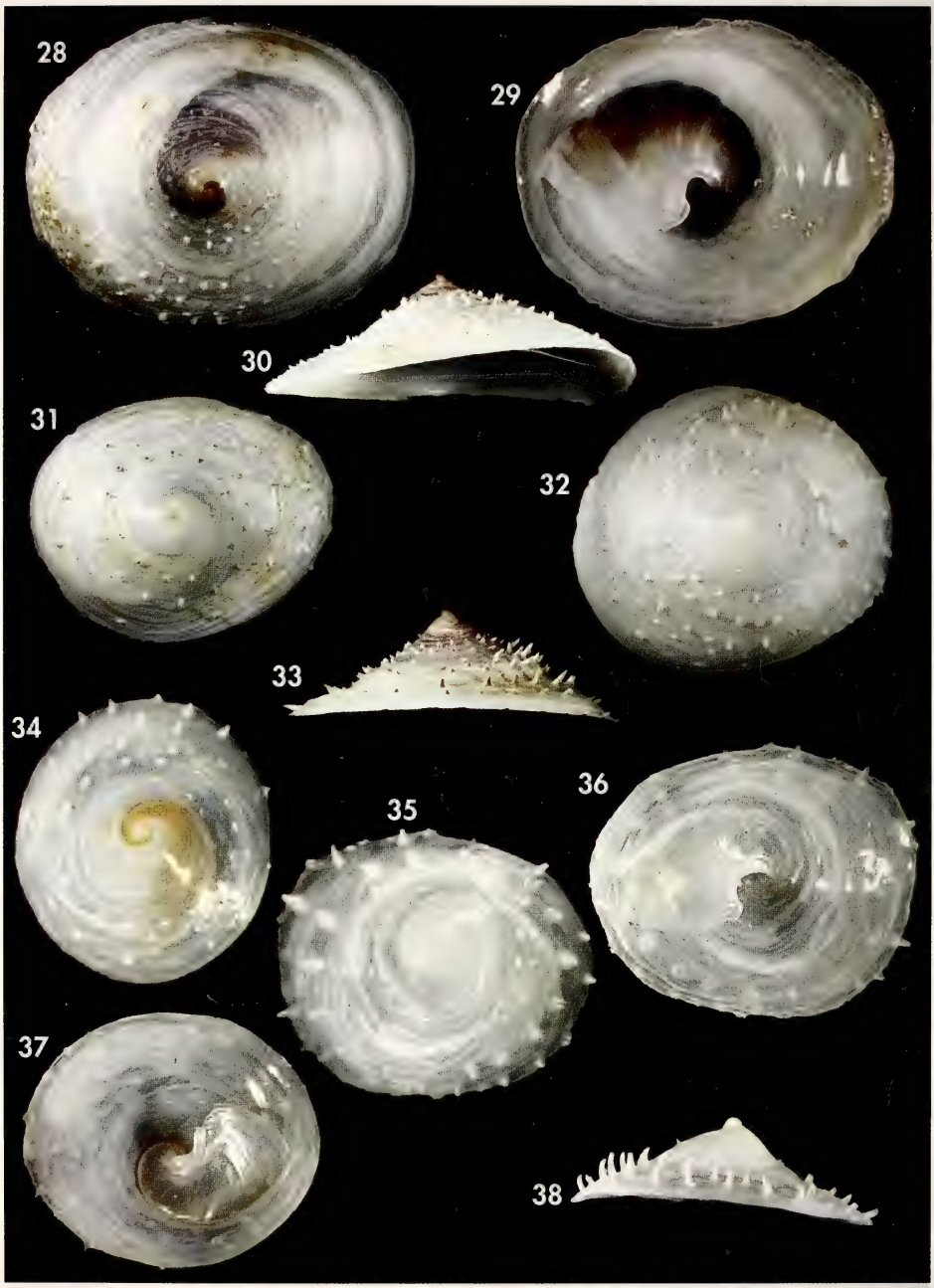


Figure 10. Juvenile shell of *Calyptraea chinensis*, 11 mm, Vigo. Figures 11-19. Protoconchs of *C. chinensis*: 11, 12: Vigo; 13: Bretagne, France; 14: Samil, Vigo; 15, 16: La Atunara, Línea de la Concepción; 17: O Grove; 18: Marbella, Spain; 19: La Atunara.

Figura 10. Concha de un juvenil de Calyptraea chinensis, *11 mm, Vigo. Figuras 11-19. Protoconchas de* C. chinensis*: 11, 12: Vigo; 13: Bretaña, Francia; 14: Samil, Vigo; 15, 16: La Atunara, Línea de la Concepción; 17: O Grove; 18: Marbella, España; 19: La Atunara.*



Figures 20-27. Protoconchs of *C. chinensis*. Fig. 20: Small protoconch, Madeira, 150 m (CFS); 21: Large protoconch, Funchal, Madeira, 80 m (CFS); 22: Large protoconch, Western Sahara, (CJH); 23: Large protoconch, La Atunara, Spain (CER). 24: Detail of the sculpture of a large protoconch, La Atunara (CER); 25: Small protoconch, Marseille, Tombant, France, 83 m (MNHN); 26: Microsculpture of a small protoconch, Tombant; 27: Large protoconch, Marseille, Tombant, 83 m (MNHN).
Figuras 20-27. Protoconchas de C. chinensis. *Fig. 20: Protoconcha pequeña, Madeira, 150 m (CFS); 21: Protoconcha grande, Funchal, Madeira, 80 m (CFS); 22: Protoconcha grande, Sáhara Occidental, (CJH); 23: Protoconcha grande, La Atunara, España (CER). 24: detalle de la escultura de una protoconcha grande, La Atunara (CER); 25: Protoconcha pequeña, Marsella, Tombant, Francia, 83 m (MNHN); 26: Microescultura de una protoconcha pequeña, Tombant, Francia; 27: Protoconcha grande, Marsella, Tombant, 83 m (MNHN).*



Figures 28-38. *C. africana* spec. nov. 28-30: Holotype, 24.4 mm, Luanda, Angola (MNCN); 31: Shell, 18.4 mm, Palmeirinhas (CER); 32: Shell, 16.6 mm, Nouadhibou, Mauritania; 33: paratype, 18.8 mm, Luanda (MNHN); 34-38: Guinea Conakry, shells (MNHN): 34: 5.0 mm; 35-36: 5.3 mm, 37-38: 5.4 mm.

Figuras 28-38. C. africana *spec. nov. 28-30: Holotipo, 24,4 mm, Luanda, Angola (MNCN); 31: Concha, 18,4 mm, Palmeirinhas (CER); 32: Concha, 16,6 mm, Nouadhibou, Mauritania; 33: Paratipo, 18,8 mm, Luanda (MNHN); 34-38: Guinea Conakry, conchas (MNHN): 34: 5,0 mm; 35-36: 5,3 mm, 37-38: 5,4 mm.*

Distribution: The species is common and widely distributed (see Figure 117) in Atlantic European and Mediterranean areas (JEFFREYS, 1882; LOCARD, 1898); United Kingdom (JEFFREYS, 1865; EALES, 1961; FRETTER AND GRAHAM, 1962, 1981; MCMILLAN, 1973; SEAWARD, 1985, 1990), Ireland (MINCHIN, MCGRATH AND DUGGAN, 1987), France (BUCQUOY, DAUTZENBERG AND DOLLFUS, 1883, LOCARD, 1892, DAUTZENBERG AND FISCHER, 1925; PERRIER, 1964; BOUCHET, DANRIGAL AND HUYGHENS, 1978; FINET, WÜEST AND MAREDA, 1992), Belgium (BACKELJAU, 1986), Spain (HIDALGO, 1917; ROLÁN, 1993; ROLÁN AND OTERO-SCHMITT, 1996), Portugal (NOBRE, 1940; MACEDO, MACEDO AND BORGES, 1999), Mediterranean (STOLFA-ZUCCHI, 1970; PARENZAN, 1970; SABELLI AND SPADA, 1977, D'ANGELO AND GARGIULLO, 1978; SABELLI *ET AL.*, 1991; RINALDI, 1991; GIANNUZZI-SAVELLI *ET AL.*, 1997; ARDUINO, LOCATELLI, ORLANDO AND REPETTO, 1995), including the Black Sea (*fide* FRETTER AND GRAHAM, 1981), North Africa (PALLARY, 1900, 1912), Morocco (PASTEUR-HUMBERT, 1962), Canary Is. and Madeira (NORDSIECK AND GARCÍA-TALAVERA, 1979), and for all European coasts (NORDSIECK, 1968; POPPE AND GOTO, 1991). It is not present in the eastern Channel and in the North Sea (FRETTER AND GRAHAM, 1981). The range is between 24° N and 56° N.

Bathymetrically, *C. chinensis* is frequently a species collected intertidally at low tide, and also circalittoral, with live collected specimens in our material from 190 m. It was recorded by the Porcupine Expedition in 1819 m (LOCARD, 1898), but only as empty shells.

The records of *C. chinensis* from South Africa (Figs. 78-80) in BARNARD (1963), KENSLEY (1973), KILBURN AND RIPPEY (1982) and STEYN AND LUSSI (1998) belong to a different species. They will be treated in a further separate paper.

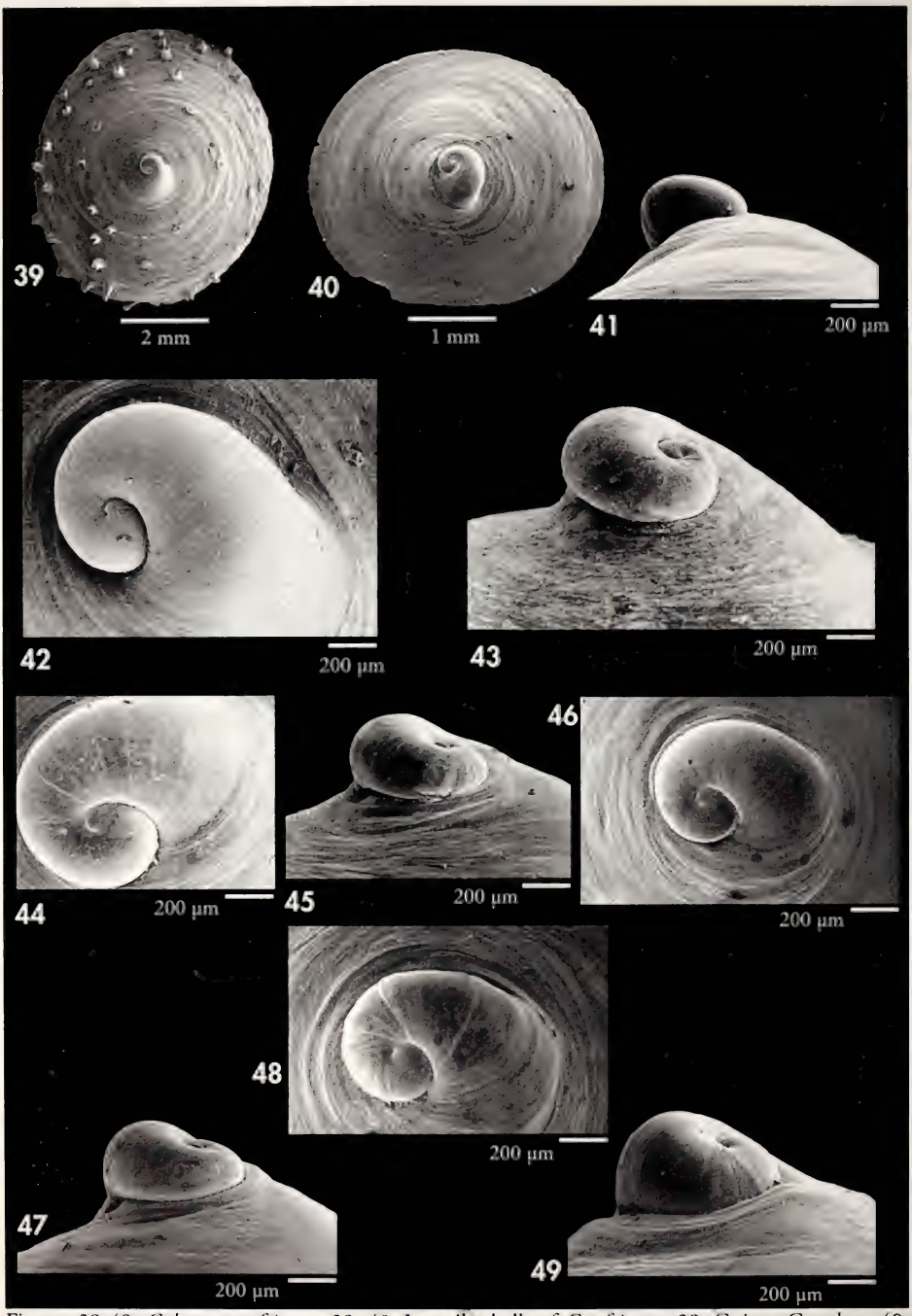
Many other records of *Calyptraea chinensis* from the West African coast correspond to two other different species, which are studied below.

Remarks: The European and north African populations are well represented by the lectotype and paralectotype at LS.

Despite the variability of the species (colour of the shell, presence or absence of spines, size, etc.) the protoconch is relatively constant in size and microsculpture in most of the shells studied. However, out of about two hundred protoconchs examined from La Atunara, seven had a larger size (870-930 µm, Fig. 23). At first it was thought that it could be a different species endemic to the Strait of Gibraltar, but no other differences were found.

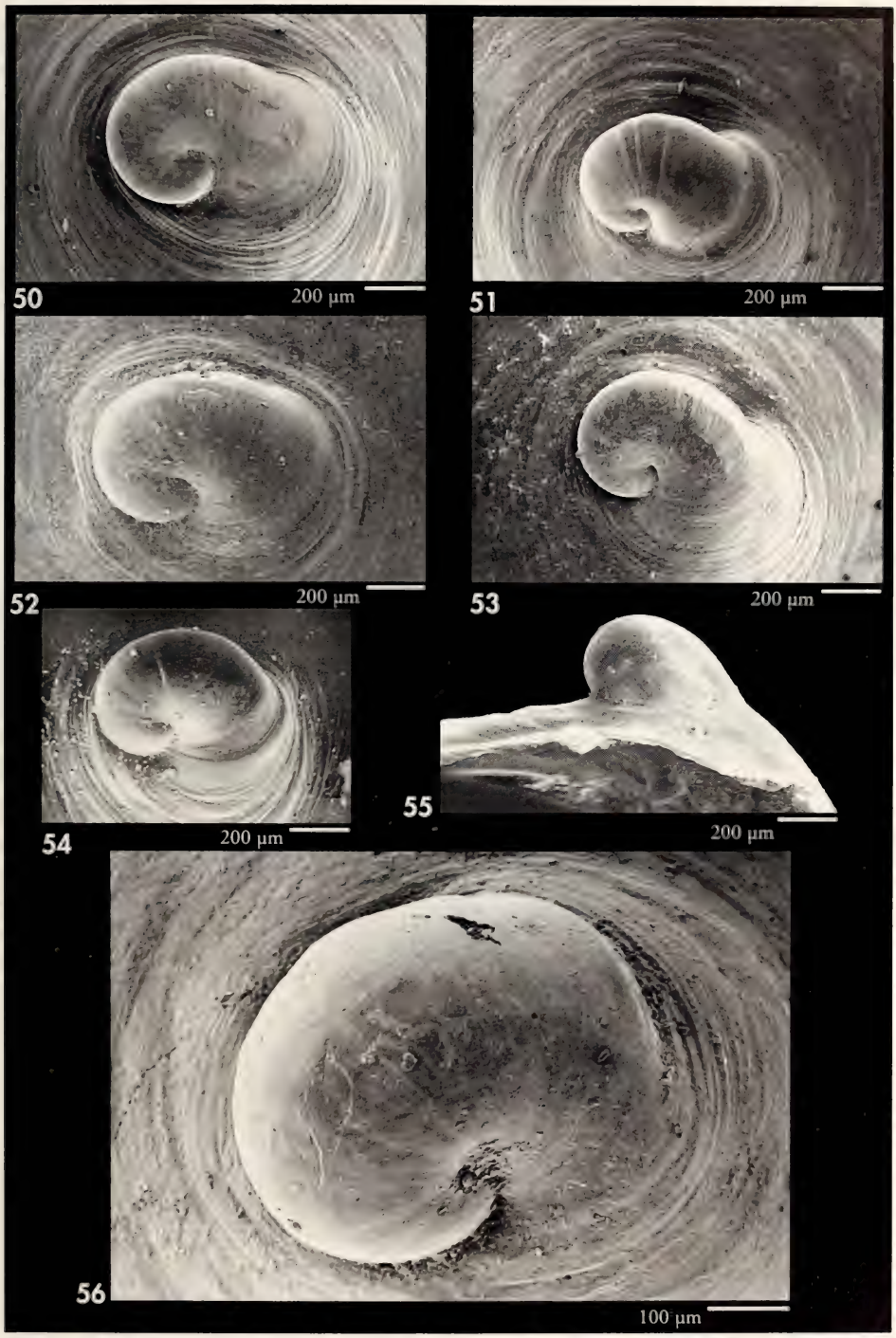
Later, we examined numerous samples from the Canaries and Madeira which had some peculiarities: they were smaller and higher than European and Mediterranean shells and more frequently brown in colour, therefore showing some similarities to *C. inexpectata* (see below). However, the protoconch was similar to those studied from France and Atlantic and Mediterranean Spain, with similar size and sculpture. Some shells of this population with larger protoconchs of almost 1 mm in diameter (Fig. 21 in comparison with Fig. 20) were found. Similarly, some large protoconchs were found in the Western Sahara population (Fig. 22). Finally, in material collected in Tombant, Marseilles, France, *Calyptraea* with both small (Fig. 25) and large (Fig. 27) protoconchs were found. In this case, the few adult shells with large protoconch were also large and very flat (Figs. 75-77), with a width/height ratio of 6.5. This is very unusual, but in considering whether they were conspecific no other differences were found.

We cannot explain why in several populations, some unusual and scarce shells have larger protoconchs which are distinct from natural variation. It is possible that, in this species with intracapsular development, some of the eggs might be used as food by viable larvae. If so, larger protoconchs would be due to greater food availability, and so, increased growth. TRONCOSO *ET AL.* (1988) mention that in a capsule with only two eggs, the size of both larvae was of 1 mm, while in another capsule



Figures 39-49: *Calyptraea africana*. 39, 40: Juvenile shells of *C. africana*; 39: Guinea Conakry; 40: Ivory Coast. Figures 41-49: Protoconchs: 41: Guinea Conakry; 42, 43: Angola; 44, 45: Ghana: 46, 47: Ivory Coast; 48, 49: Banc d'Arguin, Mauritania.

Figuras 39-49: Calyptraea africana. *39, 40: Conchas juveniles de* C. africana*; 39: Guinea Conakry; 40: Costa de Marfil. Figuras 41-49: Protoconchas: 41: Guinea Conakry; 42, 43: Angola; 44, 45: Ghana: 46, 47: Costa de Marfil; 48, 49: Banc d'Arguin, Mauritania.*



Figures 50-56. Protoconch variation in *C. africana*: 50: from Mauritania; 51: from Senegal; 52, 53: from Guinea Conakry; 54-56: from Principe.

Figuras 50-56. Variación en la protoconcha de C. africana*: 50: de Mauritania; 51: de Senegal; 52, 53: de Guinea Conakry; 54-56: de Príncipe.*

with 18 larvae, the average size was 0.6 mm.

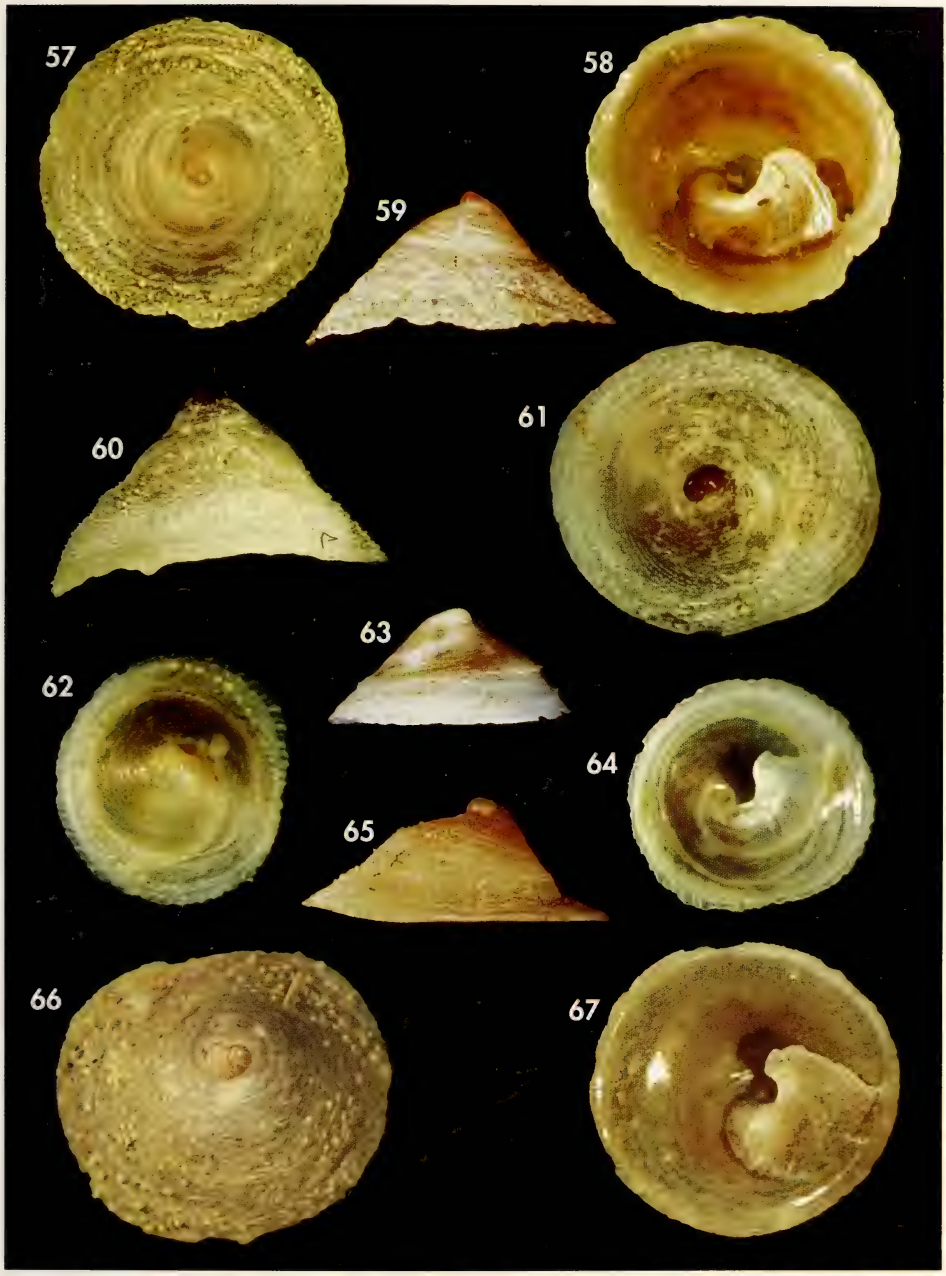
These larger protoconchs were not found in the populations examined from Galicia, Spain and Bretagne, France.

A strange shell of *Calyptraea* (Figs. 81, 82) was collected in La Palma, Canary Islands. It seems to be very different from *C. chinensis*, but it could be introduced material from ships.

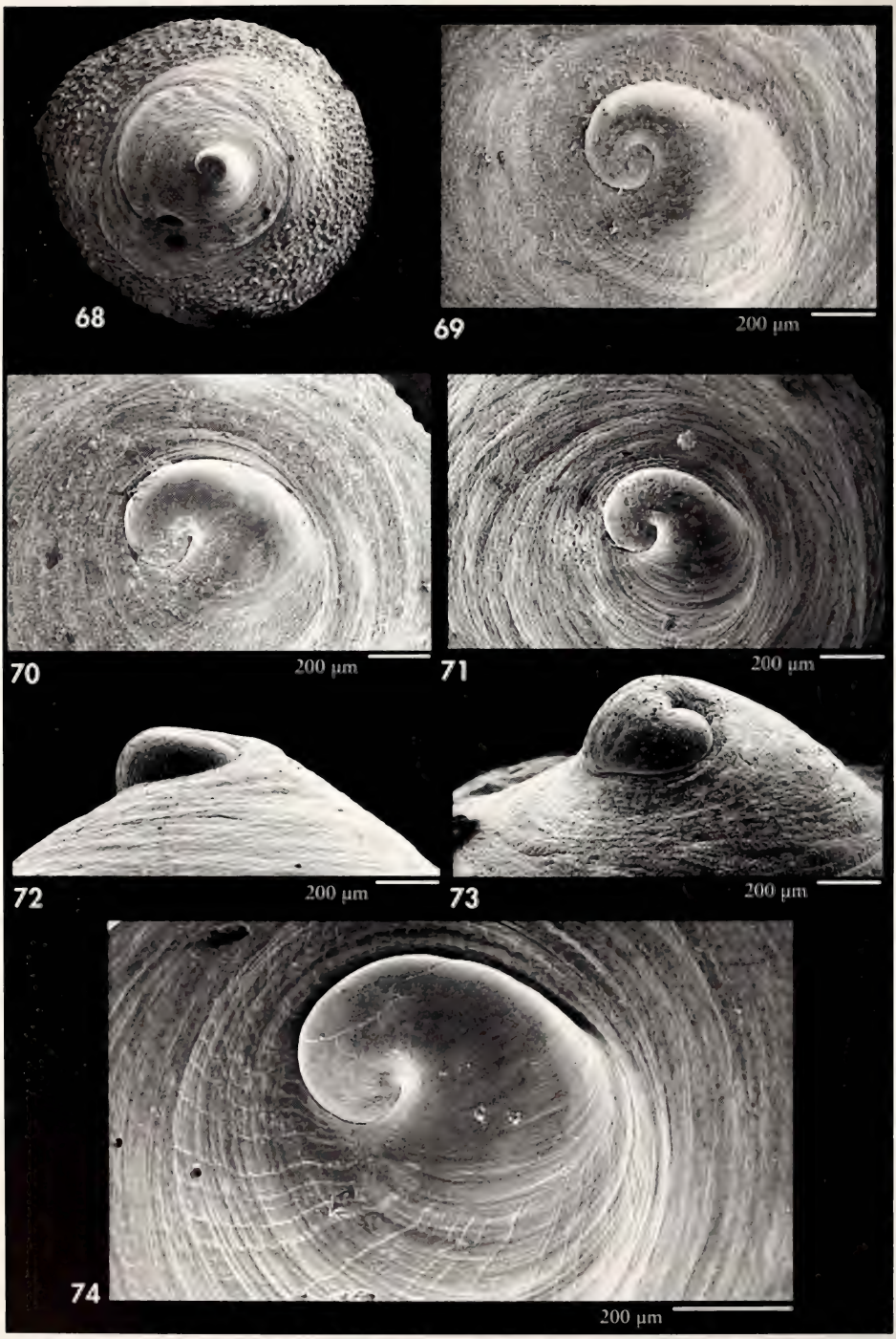
Calyptraea africana spec. nov. (Figs. 28-56, 86-88, 92, 95, 98, 101, 108-112)

Calyptraea chinensis auct. (non Linnaeus, 1758).
? *Calyptraea solida* von Martens, 1874 (non Reeve).

Type material: Holotype (Figs. 28-30) in MNCN (15.05/46621); Paratypes in the following collections: MNHN (1), AMNH (1), BMNH (1), ZSM (1), CER (30), CJH (1), CFS (1), all from Luanda (Angola).
Other material studied: Western Sahara: 17 s, Port Etienne, Mission Gruvel (MNHN). Mauritania: 12 sp, 3 miles W Kiaone 20° 02′ N 16° 22′ W, 6 m (MNHN); 6 s, 20° 10′ N 16° 30′ W, 16 m (MNHN); 15 sp, 2 s, 20° 20′ N 16° 22′ W, 10 m (MNHN); 15 c, Cansado Bay, Mission Gruvel (MNHN); 2 sp, 5 s, Nouadibhou, dredged 3 m (CER); 3 s, Baie de l′ Etoile, dredged in front Club Nautique, 4 m (CER); 155 s, 25 j, Banc d′ Arguin, intertidal sediments (CER). Senegal: 2 c, Salin La Ventite (CFS); 12 c, Thiaroye NNE Dakar, 7-8 m (MNHN); 1 c, N Cayar, 15° 12′ 5″N 15° 54′ 8″W, 25 m (MNHN); 126 s, 178 j, between Dakar and Gorée, dredging 20-40 m (CER); 3 s, Casamance, 12° 20.7′ N 16° 53.1′ W, 15 m,R/V "Louis Sauger" (MNHN); 1 s, 2 j, Cap Vert, Epave, 15 m (CER); 4 sp, M′ Bao, Dakar, 9-10 m (MNHN); 1 sp, 89 s, Almadies, Dakar, 20 m, on shells in sandy bottom (CER); 18 s, Dakar (MNHN); 2 j, Tacoma, 25 m (MNHN); 2 c, N Casamance 12° 53.8′ N 17° 03.5′ W, 19 m (MNHN); 3 sp, Region de Sine-Saloum, mangroves (MNHN); 3 c, N Casamance, Kafountine 12° 54.4′ N 17° 01.5′ W, 17 m (MNHN); 17 sp, N Casamance 12° 50.4′ N 16° 58.8′ W, 13-14 m (MNHN); 5 sp 4 s, Casamance, Zinguinchor, 3-5 m (MNHN); 17 s, S Casamance, frontier of Guinea Bissau, 4-6 m (MNHN); 1 s, Carabane Bôlon, 3-4 m (MNHN); 3 c, S Casamance, Cap Skirring, in beach sediment (MNHN). Guinea Bissau: 10 sp, Essoukkoundiak Bôlon, near frontier, 5-6 m (MNHN); 2 s, Bissagos Is., Mission L. Gain (MNHN). Guinea Conakry: 60 sp, 6 s, W Ile Knebomby N/O "André Nizery" Sedigui I, Sta. 376, 5 m, 9° 48′ N 13° 53′ W (MNHN); 12 s, W Ile Knebomby N/O "André Nizery" Sedigui I, Sta. 376, 5 m, 9° 48′ N 13° 53′ W (MNHN); 36 s, W Kaporo N/O "André Nizery" Sedigui I, Sta. 270, 6 m, 9° 36′ N 13° 38′ W (MNHN); 3 j, W Ile Quito, N/O "André Nizery" Sedigui II, Sta. 515, 26 m, 10° 00′ N 15° 43′ W (MNHN); 2 c, NW Ile Tamara, N/O "André Nizery" Sedigui I, Sta. 265, 10 m (MNHN); 2 j, SW Ile Tamara, N/O "André Nizery" Chalgui 7, Tr. 17, 18 m, 9° 28′ N 13° 53′ W (MNHN); 3 j, W Yomponi river N/O "André Nizery" Sedigui II, Sta. 724, 21 m, 10° 24′ N 15° 21′ W (MNHN); 12 j, frontier Sierra Leona, N/O "André Nizery" Chalgui 7, Tr. 6, 12 m, 9° 01′ N 13° 30′ W (MNHN); 1 s, W Kaporo N/O "André Nizery" Sedigui I, Sta. 276, 18 m, 9° 36′ N 14° 06′ W (MNHN); 2 s, W Kaporo N/O "André Nizery" Sedigui I, Sta. 275, 16 m, 9° 36′ N 14° 03′ W (MNHN); 26 s, W Morébaya river N/O "André Nizery" Sedigui I, Sta. 168, 12 m 9° 24′ N 13° 38′ W (MNHN); 5 s, 9° 40′ N 14° 05′ W 18 m (MNHN). Ivory Coast: 1 sp, 120 s, 6 j, Radiale Grand Bassam N/O Antéa Benchaci I, Sta. 12D, 30 m 5° 09.2′ N 3° 47.2′ W (MNHN); 1 s, Batrevie Beach, Sassandra (MNHN); 1 c, Abidjan (MNHN). Ghana: 52 sp, 22 s, 65 j, off Miamia, 30 m, on shell, in sandy and rocky bottom (CER). Cameroun: 1 s, Wouri Cap Nachtigal 03° 44′ N 09° 22′ E, 13 m (MNHN); 1 s, Wouri Cap Nachtigal 03° 37′ N 09° 16′ E, 40 m (MNHN); 1 sp, Victoria/Limbé, Bota, 8-10 m (MNHN). São Tomé and Principe: 1 c, Baia de Ana Chaves (MNHN); 63 s, Principe, "Calypso", 15-18 m (MNHN); 12 s, Santo Antonio, Principe, 10 m, on sandy sediments (CER). Gabon: 2 s, Cap Esterias-Pointe Idolo (MNHN); 2 s, Cap Esterias, Libreville (MNHN). Congo: 1 sp, 12 s, 12 j, Pointe Noire, Mondaine beach, 1-5 m (MNHN); 4 j, Conkouati, chalutier "Kounda", 17-19 m (MNHN); 28 sp, 10 s, Orstom Beach, Pointe Noire, 3-7 m (MNHN); 1 sp, Pointe Noire, Songolo, 6 m (MNHN). Angola: 1 sp, Cabo Ledo, Luanda, 10-40 m (MNHN); 39 sp, Corimba, Luanda, 20 m, on shells in rocky bottom (CER); 3 s, Corimba, Luanda, 10-20 m (MNHN); 10 s, Cacuaco, Bengo, 10-40 m (CFS); 4 sp, 3 s, Cacuaco, Bengo, 7 m, on shells in muddy bottom (CER); 1 s, Praia Etambar, beach sedimen (MNHN); 4 sp, 1 s, Cacuaco, Bengo, infralittoral rocks (MNHN); 6 sp, 1 s, Samba, Luanda, 2 m, on shells in sandy bottom (CER); 4 sp, Samba, intertidal (CER); 5 sp, Palmeirinhas, 15-20 m, on shells



Figures 57-64. *Calyptraea inexpectata.* 57-59: Holotype, 5.3 mm, Ile de Los, Guinea Conakry, 35 m (MNHN); 60, 61: Shell, Western Sahara, 5.8 mm (CJH); 62-64: Shell, Western Sahara, 4 mm (CJH). Figures 65-67. *Calyptraea chinensis,* form similar to *C. inexpectata*: 65: shell, 6.1 mm, Caja de Lazareto, Madeira, 60 m (CFS); 66, 67: Shell, 5.8 mm, Caja de Lazareto, Madeira (CFS).
Figuras 57-64. Calyptraea inexpectata. *57-59: Holotipo, 5,3 mm, Ile de Los, Guinea Conakry, 35 m (MNHN); 60, 61: Concha, Sáhara Occidental, 5,8 mm (CJH); 62-64: Concha, Sáhara Occidental, 4,0 mm (CJH). Figuras 65-67.* Calyptraea chinensis, *forma similar a* C. inexpectata*: 65: concha, 6,1 mm, Caja de Lazareto, Madeira, 60 m (CFS); 66, 67: Concha, 5,8 mm, Caja de Lazareto, Madeira (CFS).*



Figures 68-74: *C. inexpectata.* 68: Shell, Guinea Conakry, 5.5 mm; 69-74: Protoconchs; 69, 70: From Guinea Conakry; 71: Senegal; 72: Guinea Conakry; 73: Senegal; 74: Guinea Conakry.
Figuras 68-74: C. inexpectata. *68: Concha, Guinea Conakry, 5,5 mm; 69-74: Protoconchas; 69, 70: Guinea Conakry; 71: Senegal; 72: Guinea Conakry; 73: Senegal; 74: Guinea Conakry.*

in rocky bottom (CER); 15 s, off Luanda, 50 m, in sandy sediments (CER); 40 sp, 20 s, Mussulo, Luanda, 10-20 m (MNHN); 1 j, Lobito, intertidal (CER); 8 sp, San Antonio, Benguela, 5-10 m (MNHN); 4 s, Praia Santiago, Bengo, beach sediment (MNHN); 1 c, Mossamedes Bay, 5-10 m (MNHN);
Type locality: Luanda, Angola.
Etymology: The specific name derived from the African coasts where the species was collected.

Description: Shell (Figs. 28-40) with a patelliform shape and usually ovoid at its base (circular only in juveniles), rather fragile, transparent, smooth and shiny. It has an elevated apex, but usually the profile of the shell appears to be slightly concave, and the shell is rather depressed. Width/height ratio is about 3.3-4.5.

The protoconch may have two different forms. The first one (Figs. 40-49) has approximately 1 and $^1/_4$ whorls, with a very small nucleus which is usually 60 to 100 µm in diameter. The protoconch diameter increases quickly, to reach 10/12 times the size of the nucleus at the end. The total diameter of the protoconch is difficult to measure because the protoconch-teleoconch boundary is not evident, but may have between 700-900 µm. Seeing this protoconch laterally, we normally find that the periphery is more prominent than the teleoconch on which it is placed, resulting in a deep suture (Figs. 41, 43, 45). The second type of protoconch (Figs. 50-56) has only 1 whorl and a larger nucleus (85-157 µm) and the rate of whorl expansion is smaller (at the end the diameter of the whorl is 3-8 times that of the nucleus) and the total diameter of the protoconch is between 500-750 µm. There are no clear intergradations between these two types of protoconchs.

The teleoconch is smooth, glossy, transparent; most of the shells have sparse widely separated elevated spicules, which are not usually present in a short circle around the apex. These spicules (Figs. 28, 33-35, 38, 39, 101) are variable from one shell to another, more common in some populations and rare in others. Internally the shell is glossy.

The colour of the shell is transparent whitish. The apex is sometimes yellowish, or even dark brown, and this colour may extend along the suture; occasional shells may be almost entirely light brown. Aperture ovoid with the peristome usually at one level but sometimes curved to match the surface to which it is applied. Internally, a partition arises from the shell along a curved line running from the apex to near the aperture posteriorly.

Dimensions: Variable between populations: shells from Mauritania to Ivory Coast sometimes reach 20 mm in maximum diameter, but one exceptional shell from Dakar (MNHN) was 28.5 mm; in comparison, specimens from Angola are usually larger, about 25 mm in diameter, but can reach 28-32 mm, with a record of 35.0 mm.

Animal (described from Ghana specimens) (Fig. 95) with a rounded foot, which has two pointed extremes on the anterior border; the sole, seen with magnification, has many very small violet spots which give this tone to the whole; the head is elongate posterior to the tentacles, the snout being rather short and broad; the tentacles, milk white at the centre, sometimes with dark lines at the external border; eyes near the base in a posterior widening; the mantle has very numerous and small milk-white dots, which are grouped irregularly forming variable blotches. The penis (Fig. 98) (examined in some males of 5 mm) is bilobed at its extreme, one of the lobes being formed by two masses together; a narrow worm-like filament is present near the tip. In living males, the penis is bent dorsally towards the posterior part of the animal; the females have in this place an atrophied stub.

Seen dorsally, by transparency, a dark intestinal tract can be observed surrounding the apex which curving to the left and returning parallel to the right side. Some Angolan specimens observed live also fit this description.

Radula (Figs. 86-88, 92) with a narrow rachidian tooth with a not very prominent central cusp and up to 12

Figures 75-77. *Calyptraea* cf. *chinensis*, 17.0 x 2.6 mm, Tombant, Est du Grand Congloué, Marseille, 83 m (MNHN). Figures 78-80. *Calyptraea* aff. *chinensis*, South Africa, 19.4 y 20.2 mm (CER). Figures 81, 82. *Calyptraea* sp., La Palma, Canary Ids., 11.1 mm (CER).
Figuras 75-77. Calyptraea *cf.* chinensis, *17,0 x 2,6 mm, Tombant, Est du Grand Congloué, Marseille, 83 m (MNHN). Figuras 78-80.* Calyptraea *aff.* chinensis, *África del Sur, 19,4 y 20,2 mm (CER). Figuras 81, 82.* Calyptraea *sp., La Palma, Canarias, 11,1 mm (CER).*

Figures 83-90. Radulae of *Calyptraea*. 83: *C. chinensis*, O Grove, specimen of 12.4 mm; 84, 85: *C. chinensis* La Atunara, Línea de la Concepción, shell of 13.4 mm; 86-88: C. *africana*, Guinea Conakry, shell of 10.0 mm; 89-90: C. *inexpectata*, Tacoma, Dakar, shell of 12.0 mm.

Figuras 83-90. Rádulas de Calyptraea. *83:* C. chinensis, *O Grove, ejemplar de 12,4 mm; 84, 85:* C. chinensis *La Atunara, Línea de la Concepción, concha de 13,4 mm; 86-88:* C. africana, *Guinea Conakry, concha de 10,0 mm; 89-90:* C. inexpectata, *Tacoma, Dakar, concha de 12,0 mm.*

smaller cusps on each side. Lateral tooth slightly prominent and with a narrow cusp in the confluence of the internal and the external edges; internal edge with numerous and irregular cusps (usually up to 11), while the external edge has more numerous cusps (22-30). Marginal teeth elongate, curved, sharply pointed; the internal with two borders with up to 20 cusps on the internal border and less on the external (4-6), while the external teeth have up to 15 cusps on its internal part.

The spawn is formed by numerous ovoid elongate capsules (Fig. 108-111) which are fixed to the substrate and sat upon by the animal. On some occasions, when the animal was removed from the substrate, some capsules remained around the foot (Fig. 110). In the larger Angolan specimens, the spawn was composed of up to 48 capsules, with 57/90 eggs in each. In the case of a small specimen of 12 mm, there were 18 capsules with 13 - 35 eggs of 170-260 µm diameter. In Ghana specimens, animals between 8-13 mm had 17-26 capsules/each and between 10-30 eggs per capsule, with an average egg diameter of 166 µm. At hatching, the shells already have the juvenile morphology (Fig. 112).

Distribution: The shells mentioned as *Calyptraea chinensis* from West Africa or from countries in this area are probably best referred to the present species: DAUTZENBERG (1912), KNUDSEN (1950) and NICKLÉS (1950) recorded it in several localities; NICKLÉS (1947) also for Senegal; LAMY (1923) for Bissagos Islands; BUCHANAN (1954) for Ghana; TOMLIN (1923) and FERNANDES AND ROLÁN (1993) for São Tomé and Principe; BERNARD (1984) for Gabon; COLLINGTON (1960) for Congo; GOFAS, AFONSO AND BRANDÃO (1985) and ROLÁN AND RYALL (1999) for Angola.

In our material, the species is represented by shells and specimens collected from south of Western Sahara/Mauritania (21.5° N) down to Angola (15° S), including the Gulf of Guinea islands of São Tomé and Principe (Fig. 117). It was not collected in the Cape Verde Islands or in Annobon.

This species usually occurs in shallow water. The deepest live-collected specimens from the Ghana area are from 20-30 m, but are usually found in shallower waters in Mauritania, Senegal, Guinea Conakry and Angola.

Discussion: The new species *C. africana* differs from *C. chinensis* in numerous characters (Table I). The most important are: *C. chinensis* is usually smaller, more rounded in form and rarely appearing ovoid; the shell is more solid at a similar size, not transparent, more variable in colour and the external surface is not glossy; the projections on the external surface, when they are present, are denser and not very prominent; the protoconch has a larger nucleus, larger spire (1 $^{1}/_{4}$ versus $^{3}/_{4}$ - 1), spiral rows of micro-undulations, and seen from the side the protoconch is more distinct from the teleoconch by a deeper suture. The penis is also different, *C. chinensis* has an elongate penis expanded into two lobes at its apex like a spoon with a thumb in opposition, while *C. africana* is bilobed and has on one side two masses together and a narrow filament. The spawns are also different, with capsules more elongate in *C. africana* and more spherical sometimes slightly ovoid in *C. chinensis*; the eggs are smaller in *C. africana* than in *C. chinensis*.

KNUDSEN (1950) probably examined the spawn of *C. africana* because one of the populations mentioned was collected at 17 m, in the middle of the distribution of this species. The other two populations were probably from a different species because *C. africana* has not been collected alive at 34-50 m.

The presence of two kinds of protoconchs with different characters without intergradations made us suspect that we could be working with two different species. This could correspond to two sympatric sibling species. Furthermore, the most common type of protoconch, the larger one, is the only form observed in Angolan populations; the smaller one appears more frequently in the Principe population and also in shells from Ghana, Ivory Coast, Guinea Conakry and Senegal, but is always present in

Table I. Differences in characters of the shell, protoconch, radula and spawn of *Calyptraea chinensis, C. africana* spec. nov. and *C. inexpectata* spec. nov.
Tabla I. Diferencias entre los caracteres de la concha, protoconcha, rádula y puesta de Calyptraea chinensis, C. africana *spec. nov. y* C. inexpectata *spec. nov.*

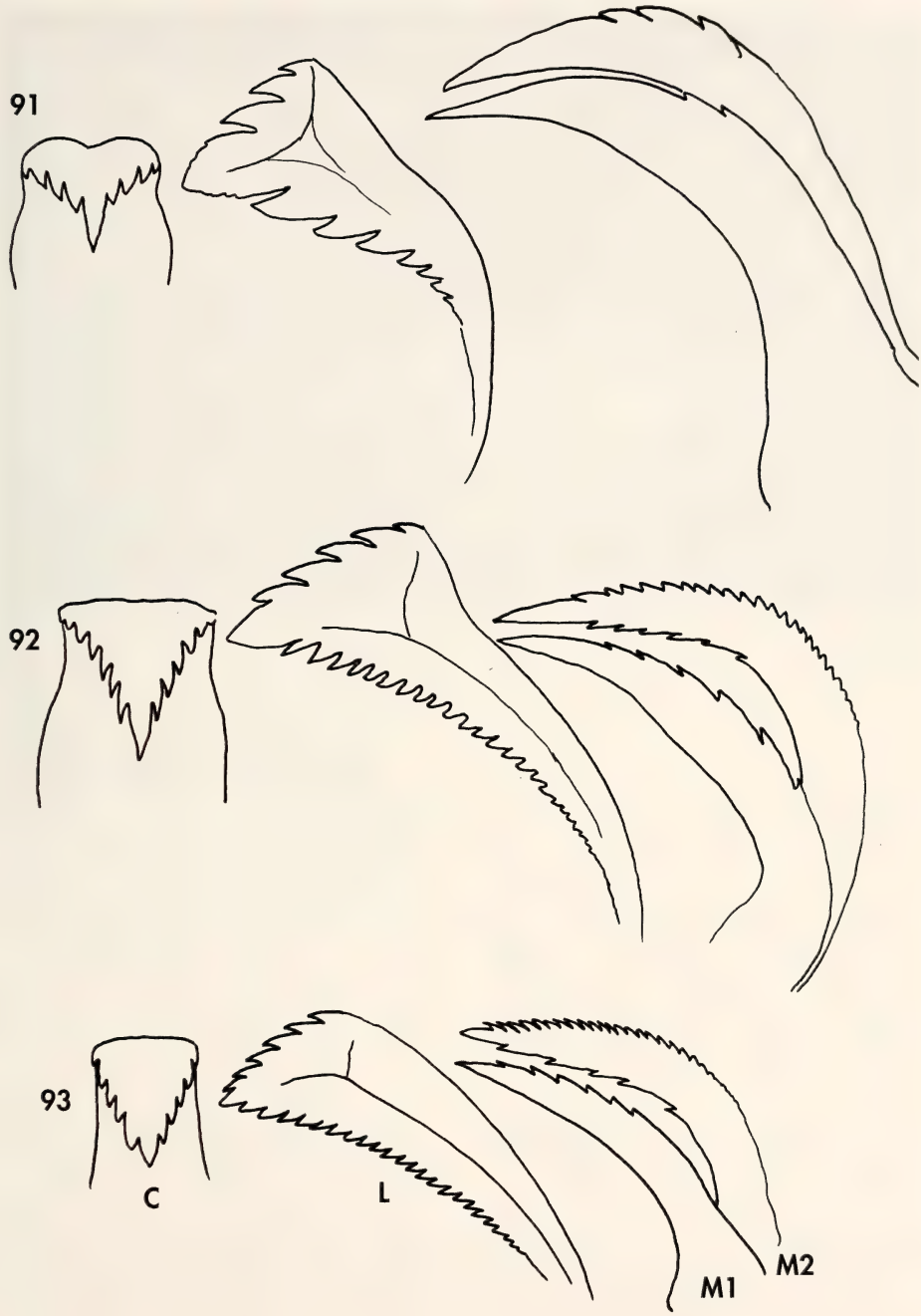
	C. chinensis	*C. africana* spec. nov.	*C. inexpectata* spec. nov.
Profile of the shell	hooked	tendency to be concave	tendency to be convex
Form of the aperture	rounded	ovoid in adult	rounded irregularly
Border of the shell aperture	uniform	uniform	frequently undulate or irregular
Transparency of the shell	no	yes	no
Shell width / height ratio	2.5-3.1	3.3-4.5	1.8-2.4
Most common coloration	white	white or white with the apex yellowish or brown	totally white or brown
Other possible colorations	pink, brown, violet	subsutural brown band	apical brown
External sculpture	usually smooth, sometimes with numerous prominences	usually smooth, sometimes with very elevated and separate prominences	sometimes wrinkled, frequently numerous scarcely elevated prominences like scales
Usual shell diameter	up to 15 mm	up to 25 mm	up to 7.5 mm
Maximum diameter	25.0 mm	35.0 mm	12.0 mm
Protoconch: diameter	600-741 µm	form 1: 700-900 µm form 2: 500-750 µm	471-614 µm
Protoconch: diameter of the nucleus	194-230 µm	form 1: 60-100 µm form 2: 85-157 µm	142-215 µm
Protoconch: number of whorls	$^3/_4$ - 1	form 1: 1 $^1/_4$ form 2: 1	1
Diameter nucleus/diameter at the end of the protoconch ratio	3-3.5	form 1: 10-12 form 2: 3-8	2.7-4.5
Elevation of the protoconch from the teleoconch	slightly elevated and suture not deep	elevated with deep suture	slightly elevated and suture not deep
Protoconch sculpture	spiral bands of axial striae	no	no
Sculpture at the beginning of teleoconch	no	no	spiral grooves
Spawn: capsules	spherical scarcely ovoid	ovoid	unknown
Eggs size	340-490 µm	160-270 µm	unknown
Animal mantle	transparent with some white spots grouped in the border	transparent with irregular white spots	transparent with very evident radiating milk white lines at the border
Penis	bilobed with distally a opposing thumb on a spoon	bilobed like two fused eggs, with other prominence and a fine worm like filamente	elongate, straight, with two angulations
Depth limits of the live collected specimens	0 – 190 m	3 – 30 m	25-53 m

small numbers (about 10%). For this reason, we undertook a detailed study to find other differences which can usually be found in two sibling species. In the present case, the comparison of animals, shell characters, radula, etc. did not show other differences and it was not possible to prove that these different protoconchs were the result of sampling two species. For the time being we decided to consider the shells with these two kinds of protoconchs as conspecific. The holotype is a shell with the large type of protoconch, from Luanda, Angola, an area where the second type of protoconch has never been found. Currently there is no explanation for this difference, and it is expected that the future study of DNA will provide additional information.

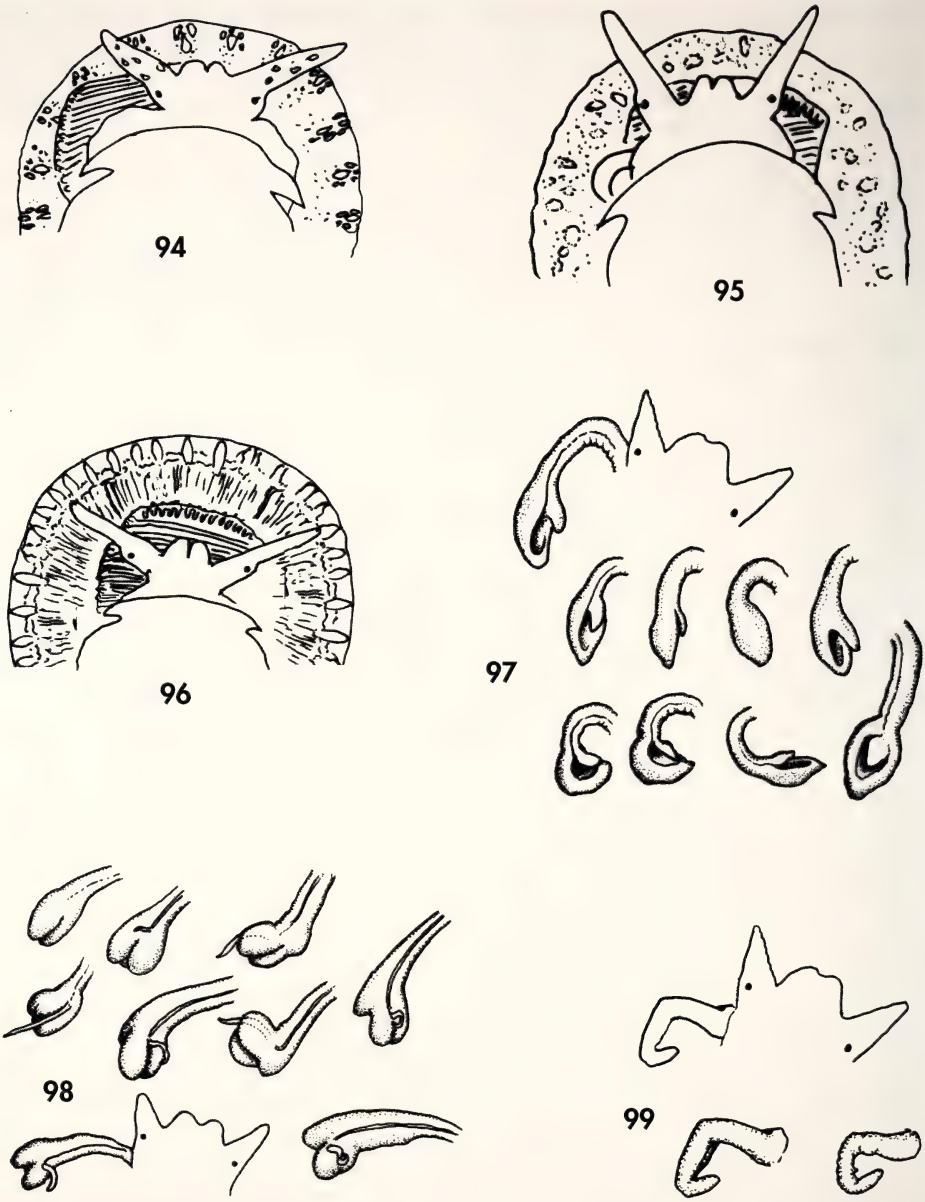
Calyptraea inexpectata spec. nov. (Figs. 57-74, 68-74, 89, 90, 93, 96, 99, 102)

Type material: Holotype (Figs. 57-59) deposited in MNHN with 1 paratype from type locality; 10 paratypes more, from W Kaporo, Sta. 275, 16 m, 9° 36′ N 14° 03′ W; 2 more from W Pte. Goro, Sta. 544, 41 m, and 1 more from W Cap Verga, Sta. B5CH, 20 m, 10° 12′ N 13° 06′ W, all them from N/O "André Nizery" Sedigui I and II. Other paratypes, from 40 m between Dakar and Gorée, in the following collections: MNCN (15.05/46622) (1), AMNH (1), BMNH (1), ZSM (1), CER (68), CJH (1), CFS (1).
Other material examined: Western Sahara: 2 s, 23° 05′ N 16° 00′ W, 27-36 m (CJH); 1 sp, (CJH); 1 s, 22° 35′ N 16° 58′ W, 86 m (CJH); 2 s, 23° 05′ N 16° 00′ W, 25-50 m, (CJH); 1 sp, (CJH); 3 s, 22° 35′ N 16° 58′ W, 86 m (CJH). Mauritania: 1 s, 17° 42′ N 16° 12′ W, 46 fms, (CJH); 8 s, 17° 22′ N 16° 17′ W, dredged at 70-100 m (CJH); 2 s, (MNHN); 1 s, N/O N′ Diago 18° 12′ N 16° 20′ N, 50 m (MNHN); 1 s, N/O N′ Diago 19° 12′ N 16° 40′ N, 47 m (MNHN); 3 s, N/O N′ Diago 19° 06′ N 16° 34′ N, 37 m (MNHN); 1 s, N/O N′ Diago 17° 42′ N 16° 25′ N, 50 m (MNHN); 4 s, N/O N′ Diago 17° 18′ N 16° 26′ N, 76 m (MNHN); 1 s, N/O N′ Diago 17° 36′ N 16° 23′ N, 65 m (MNHN); 1 s, N/O N′ Diago 19° 00′ N 16° 30′ N, 31 m (MNHN); 1 c, 17° 17′ N 16° 30′ W, 85 m (MNHN); 1 s, 17° 45′ N 16° 23′ W, 300-600 m (CFS). Senegal: 1 sp, 12 s, Tacoma, 25 m (CER); 2 s, 3 j, Cap Vert, Epave, 15 m (CER); 5 sp, 225 s, SW Ile Madeleine, Dakar, 48 m (MNHN); 3 j, SW Madeleines, Dakar, 455-465 m (MNHN); 4 c, Dakar 14° 23′ 5″N 17° 24′ 5″W, 65-70 m (MNHN); 53 s, Gorée, 20 m (CFS); 3 sp, dredged near Gorèe, 15-25 m (CER); 20 s, S Gorée, Dakar, 33-42 m (MNHN); 16 s, 6 j, S Gorée, 98 m (MNHN); 6 s, Dakar 14° 27′ N 17° 33′ W, 145-200 m (MNHN); 13 j, Dakar, 6 m (CER); 8 s, 8 j, Dakar, 20 m (CER); 15 s, E Gorée, 20 m (MNHN); 49 s, S Gorée, "G. Treca", 65 m (MNHN); 1 sp, 14 s, 8 j, Tacoma, 20-25 m (CER); 5 s, Delta of Saloum, (CJH); 1 sp, N Casamance 13° 01.8′ N 17° 25.5′ N "Louis Sauger", 53 m (MNHN); 1 s, Casamance, 12° 20,7′ N 16° 53.1′ W, R/V "Louis Sauger", 15 m (MNHN); 2 s, N Casamance 12° 32′ N 17° 28.8′ N "Louis Sauger", 45 m (MNHN); 18 sp, N Casamance 12° 46.9′ N 17° 29.9′ N "Louis Sauger", 45 m (MNHN); 39 s, 40 j, between Dakar and Gorée, dredged at 20-40 m (CER). Guinea Conakry: 1 s, W Kaporo N/O "André Nizery" Sedigui I, Sta. 276, 18 m, 9° 36′ N 14° 06′ W (MNHN); 5 c, 3 j, Baie de Sangarea N/O "André Nizery" Sedigui II, Sta. B12-13CH, 35 m, 9° 42′ N 15° 33′ W (MNHN); 1 s, W Ile Tannah, Sedigui I, Sta. 84. 9° 12′ N 13° 49.5′ W, 33 m (MNHN); 1 s, (CJH); 1 s, 09° 56′ N 15° 58′ W, 36 m (CJH). Benin: 2 s, Ouidah 06° 10′ N 02° 05′ E, 200 m (MNHN).
Type locality: Île de Los, Guinea Conakry, Expedition Sedigui I, Sta. 262, 9° 30′ N 13° 59′ W, 35 m.
Etymology: The specific name alludes to the fact that this species appeared while comparison between *C. chinensis* and *C. africana* was being made, and was not expected when the study was begun.

Description: Shell (Figs. 57-64, 68) with a patelliform shape and usually rounded at its base, relatively solid, not transparent, smooth but not shiny, sometimes with a rough surface. It has an elevated apex, and the profile usually shows a right or slightly convex line. The shell is not depressed. Ratio between width and height is between 1.8 and 2.4. The protoconch (Figs. 69-74) has approximately 1 whorl, with a nucleus of 142-215 µm in diameter. From this nucleus, the protoconch diameter increases quickly, to about 2.7-4.5 times that of the nucleus. The total diameter of the protoconch in the mater-

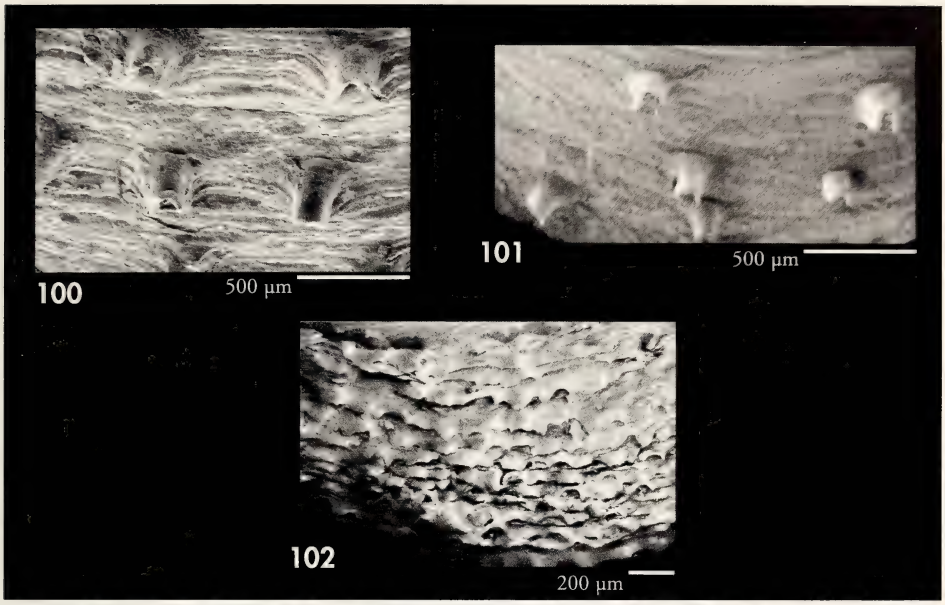


Figures 91-93: Drawing of the radulae of *Calyptraea*. C: central tooth; L: lateral tooth; M1, M2: marginal teeth. 91: *C. chinensis*, specimen of 13 mm, Vigo, Spain; 92: *C. africana*, specimen of 11 mm, Luanda, Angola; 93: *C. inexpectata*, specimen of 12 mm, Tacoma, Dakar.
Figuras 91-93: Dibujo de los dientes radulares de Calyptraea. *C: diente central; L: diente lateral; M1, M2: diente marginal. 91:* C. chinensis, *ejemplar de 13 mm, Vigo, España; 92:* C. africana, *ejemplar de 11 mm, Luanda, Angola; 93:* C. inexpectata, *ejemplar de 12 mm, Tacoma, Dakar.*



Figures 94-96: Diagram of the animal of *Calyptraea*. 94: *C. chinensis*, Vigo, Spain; 95: *C. africana*, Miamia, Ghana; 96: *C. inexpectata*, Dakar, Senegal. Figures 97-99. Penis of *Calyptraea*: 97: *C. chinensis* upper row, from Vigo, Spain, specimens; lower row: first 2, from O Grove, Galicia, Spain; the last 2 from La Atunara, Spain; 98: *C. africana*: upper row and first of the second from Miamia, Ghana; the rest form Luanda, Angola; 99: *C. inexpectata*, Dakar, Senegal.

Figuras 94-96: Esquema del animal de Calyptraea. *94:* C. chinensis, *Vigo; 95:* C. africana, *Miamia, Ghana; 96:* C. inexpectata, *Dakar, Senegal. Figuras 97-99. Pene de* Calyptraea*: 97:* C. chinensis *fila superior, ejemplares de Vigo, España; fila inferior: los 2 primeros, de O Grove, Galicia, España; los 2 últimos, de La Atunara, España; 98:* C. africana*: fila superior y primero de la segunda fila de Miamia, Ghana; el resto de Luanda, Angola; 99:* C. inexpectata, *Dakar, Senegal.*



Figures 100-102. Sculpture of the shells. 100: *Calyptraea chinensis*; 101: *C. africana*; 102: *C. inexpectata*.
Figuras 100-102. Escultura de las conchas. 100: Calyptraea chinensis*; 101:* C. africana*; 102:* C. inexpectata.

ial studied was 471-614 µm. In lateral view the periphery of the protoconch is not very prominent from the teleoconch, and the suture is shallow (Figs. 72, 73). The protoconch surface lacks any sculpture except for growth lines.

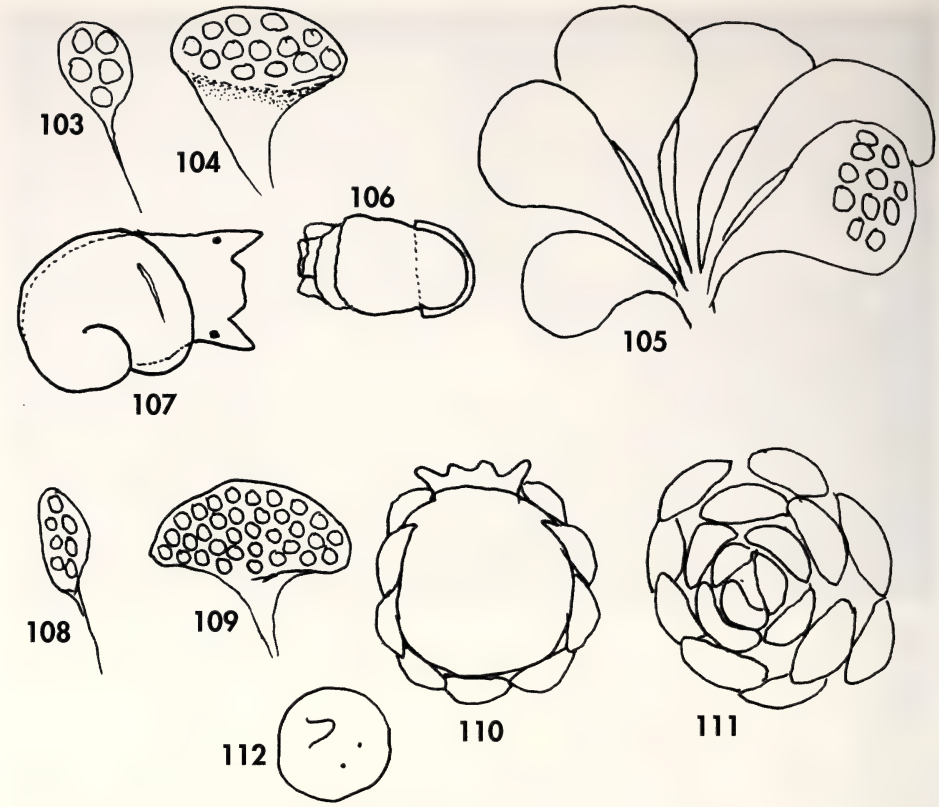
The teleoconch has at its beginning numerous growth lines crossed by spiral grooves (Figs. 69, 70, 74) which radiate from the protoconch; the teleoconch is opaque and not glossy, and in many shells spiral striae exist. Most of the shells have numerous wavy scales (Fig. 102), closer between them, which are usually absent in a short circle around the apex (Fig. 68). These scales are variable among populations, and are sometimes rare. Internally the shell is glossy.

The colour of the shell is whitish or brown, usually uniform, each shell with a single colour. Aperture rounded with the peristome frequently irregular, perhaps due to irregular substrate. Internally, a partition arises from the shell along a curved line running from the apex to near the aperture posteriorly.

Dimensions: in most of the populations the shells are only 5-7 mm, but in some, the largest shells reach 12 mm in maximum diameter.

The animal (Fig. 96), examined in five specimens (three females and 2 males) of the Dakar population, is whitish translucent with numerous milk-white opaque spots on the tentacles, the lips and the body. The mantle has very marked milk-white radiated fusiform blotches which reach the border. All of them are fused into a continuous line a little below the border, but continue towards the interior in other finer lines. The buccal lips have a darker tone. The males are very small (3 mm diameter). The penis observed in the two males studied (Fig. 99) is behind the right tentacle and difficult to see because it is in a dorsal position; it is cylindrical and simple, having two angulations.

Radula (Figs. 89, 90, 93) with a rachidian tooth narrow and with the central cusp not very prominent and up

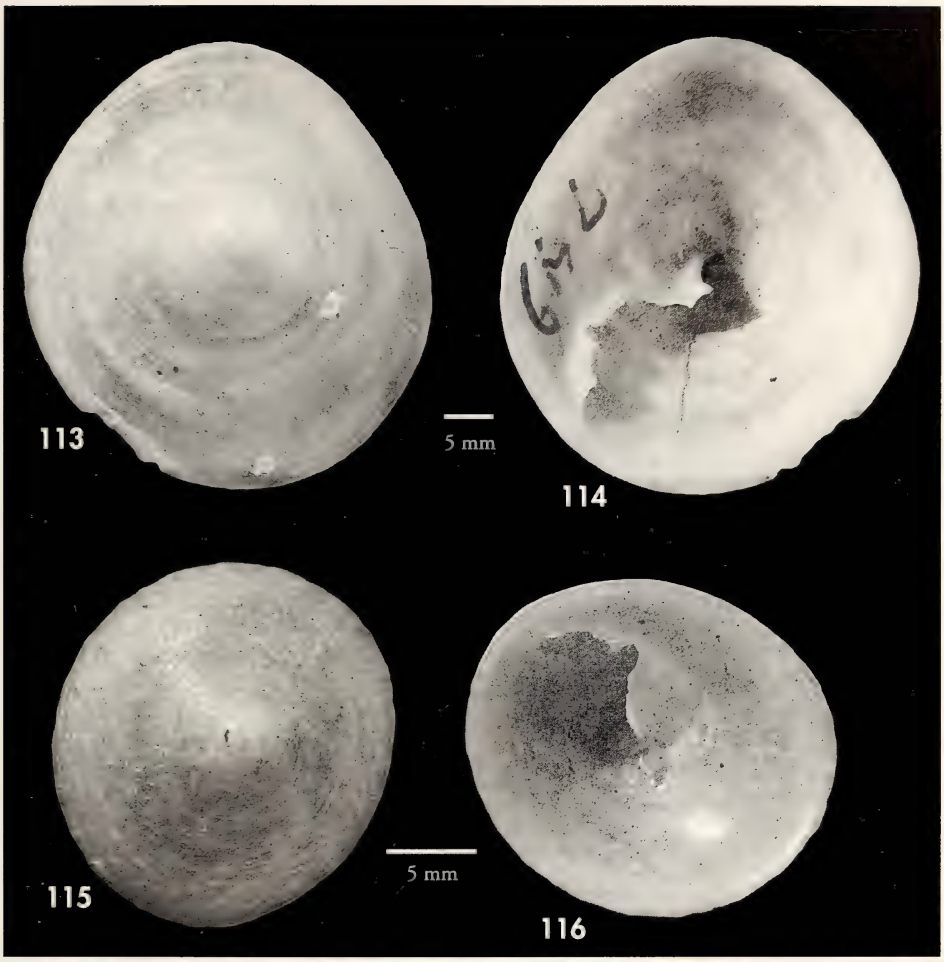


Figures 103-112. Spawn and larval specimens of *Calyptraea*. Figures 103-107. *C. chinensis* from Vigo. 103, 104: egg capsules; 105: group of egg capsules. 106, 107: larval specimens after the veliger period. Figures 108-112. *C. africana* from Miamia, Ghana. 108, 109: egg capsules; 110: position of the eggs with the animal on the substrate; 111: spawn removed the animal; 112: larval specimen.
Figuras 103-112. Puesta y ejemplares larvarios de Calyptraea. *Figuras 103-107.* C. chinensis *de Vigo. 103, 104: cápsulas; 105: grupo de cápsulas; 106, 107: ejemplares larvarios posteriores a la etapa velígera. Figuras 108-112.* C. africana *de Miamia, Ghana. 108, 109: cápsulas; 110: posición de las cápsulas con el animal sobre el sustrato; 111: puesta una vez retirado el animal; 112: ejemplar larvario.*

to 5 smaller cusps at each side. Lateral teeth with a cusp in the confluence of the internal and the external edges which has the same size as those in the internal edge (usually up to 5) while the external edge has more numerous cusps (16-20). Marginal teeth elongate, curved, sharply pointed and the internal ones with up to 22 cusps on the internal border and 4-6 on the external. The external teeth have up to 6 cusps on its internal part.

The spawn of *C. inexpectata* was probably examined by KNUDSEN (1950), because two of the populations mentioned in this work are from 34 and 50 m, which is the usual depth for this species. He mentioned 23 and 28 capsules per brood, with 9-29 eggs per capsule and a total between 239-274 eggs. Only one spawn of this species could be examined, but in poor conditions to count and measure capsules and eggs.

Distribution: *C. inexpectata* is known from Western Sahara (23° N) and Mauritania to Benin (6° N) (Fig. 117). It was not found in Ghana in spite of the many samples obtained from this country.



Figures 113-116. Types of *Calyptraea chinensis* in LS: 113, 114: Lectotype; 115, 116: Paralectotype.
Figuras 113-116. Tipos de Calyptraea chinensis *en LS: 113, 114: Lectotipo; 115, 116: Paralectotipo.*

This species has not been found in shallow water. The living material has been collected from 25 to 53 m, and empty shells from up to 600 m.

Discussion: *C. inexpectata* is different from the other species mentioned in the present work. The differentiating characters are listed in Table I, but they will be commented as follow:

C. chinensis is usually larger; the sculpture (when it is present) is more prominent and less dense; the shell width/height ratio is larger (2.5-3.1 vs. 1.8-2.4); the protoconch is rather similar, but a little larger and also the nucleus diameter is slightly larger, showing microsculpture which does not exist in *C. inexpectata*. The beginning of the protoconch in *C. chinensis* lacks the grooves present in *C. inexpectata*. The radula has fewer cusps on the lateral tooth, and the cusp in the confluence of the internal and external borders is larger; also, there are fewer cusps on the marginal teeth; the central tooth is wider.

C. africana has a larger shell, usually about 25 mm in diameter, and up to 28-32 mm, while *C. inexpectata* seldom reaches

Figure 117. Distribution range of *C. chinensis*, *C. africana* and *C. inexpectata*.
Figura 117. Área de distribución de C. chinensis, C. africana *y* C. inexpectata.

12 mm. Also, *C. africana* is ovoid in its aperture, more depressed, more transparent, shiny, with external sculpture (when present) formed by isolated and raised spicules, in contrast to the scaly concentric lines of *C. inexpectata*. The protoconch of *C. africana* is larger (in the commonest form), with a smaller nucleus, more prominent and deeper suture between the protoconch and the teleoconch. The radula has more cusps and some different details, such as the larger cusp in the confluence of the external and internal edges of the lateral tooth.

These two species co-occur, in Sahara-Mauritania, Senegal and Guinea Conakry. *C. africana* was collected in beach sediments at a few meters depth, while *C. inexpectata* was collected in deeper water, at least 30-50 m, but sometimes deeper (shells collected frequently in 70-100 m, up to 600 m), where no shells of *C. africana* were found.

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Sinonimias
Doris limbata Cuvier, 1804, *Ann. Mus. H. N. Paris*, 4 (24): 468-469 [Localidad tipo: Marsella].
Doris nigricans Otto, 1823, *Nov. Act. Ac. Caes. Leop. Car.*, 10: 275.

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Synonyms
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ÍNDICE

Iberus 22 (2) 2004

NAGEL, K-O. Observations on the reproductive period of the freshwater mussel *Potomida littoralis* (Unionidae)
Observaciones sobre el periodo reproductor del mejillón de agua dulce Potomida littoralis *(Unionidae)* 1-8

MARTÍNEZ-ORTÍ, A., APARICIO, Mª T. Y ROBLES, F. La malacofauna de la Sierra de Alcaraz (Albacete, España)
The molluscan fauna of the Alcaraz mountains (Albacete, Spain) 9-17

PÉREZ-QUINTERO, J. C., BECH TABERNER, M. Y HUERTAS DIONISIO, J. L. Los moluscos de las aguas continentales de la provincia de Huelva (SO España)
Freshwater Molluscs of Huelva Province (SW Spain) 19-31

ÁVILA, C., FONTANA, A., ESPOSITO, M., CIAVATTA, Mª L. AND CIMINO, G. Fatty acids of Antarctic gastropods: distribution and comparison with Mediterranean species
Ácidos grasos en gasterópodos antárticos: distribución y comparación con especies mediterráneas 33-44

DELL'ANGELO, B., ANSEEUW, B., TERRYN, Y. AND BONFITTO, A. Why are beached *Acanthochitona fascicularis* (Linnaeus, 1767) (Mollusca: Polyplacophora) from Italy missing their dorsal girdle elements?
¿Por qué los ejemplares de Acanthochitona fascicularis *(Linnaeus, 1767) (Mollusca: Polyplacophora) encontrados en una playa de Italia pierden los elementos del perinoto?* 45-49

ROLÁN, E. The genus *Calyptraea* (Gastropoda, Caenogastropoda, Calyptraeidae) in the East Atlantic
El género Calyptraea *(Gastropoda, Caenogastropoda, Calyptraeidae)en el Atlántico oriental* 51-79

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